Factors affecting the quality of *Acacia senegal* gums

Thesis submitted in accordance with the requirements of the University of Chester for the degree of Doctor of Philosophy

by

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DECLARATION

The material being presented for examination is my own work and has not been submitted for an award of this or another HEI except in minor particulars which are explicitly noted in the body of the thesis. Where research pertaining to the thesis was undertaken collaboratively, the nature and extent of my individual contribution has been made explicit.

Signed ……(Candidate)

Date……

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Abstract

Gum arabic is a natural gummy exudate from *acacia* trees and exhibits natural built-in variations commonly associated with hydrocolloids. This study is concerned with the determination of factors which could influence its properties and functionality. These factors include origin (location, soil type, rainfall), different collections, age of the trees and storage condition. Previous studies acknowledged the influence of some of these factors but somehow lack providing definitive answers to questions being asked by the end user and required for the development of Gum arabic industry in Sudan. Local knowledge as well as the various stages of gum collection and processing were reviewed in order to provide a clear background and the justification for the experimental design. In this study samples were collected from six plantations located in the west and east regions in Sudan. Samples were collected from trees of different age (5, 10, 15 and 20 years old) and also from different picking interval (1-4). Each sample was divided into three portions (UK, Khartoum and Port Sudan) and stored for 5 years in order to determine the effect of the respective location. Various analytical parameters (% loss on drying, Optical rotation, % protein, intrinsic viscosity, molecular weight and molecular weight distribution) were measured to fully characterise the gum samples and to determine their functionality (emulsification). The results obtained for all samples were consistent with those previously reported in the literature (see Chapter 4). The only exception, identified in a number of samples from the western region, is the high proportion (~30%) of high molecular weight fraction termed arabinogalactan-protein complex (AGP). The results clearly demonstrated significant variations between plantations located in western region compared with the eastern region. However, the variations between the plantations within the same region are statistically not significant. High values of % protein, viscosity, M_w and % AGP were obtained from the 1st pick, from both regions, and then significantly decreased thereafter to the fourth pick. Samples from west region in Sudan, from 1st and 2nd pick and from tree age (15) years gave the highest viscosity, molecular weight, % AGP and superior emulsification performance compared to other samples from different tree ages. The regression statistical analysis for the physiochemical properties correlation with emulsification performance demonstrated the role of % AGP to be the most influential factor followed by viscosity. The major finding of this study is the effect of storage condition on the properties and functionality of *Acacia senegal*. An increase in the molecular weight for all stored samples (for 5 years) irrespective of region was evident and statically significant. However, this increase was more prominent for samples from the western region compared to the eastern region. The AGP fraction was increased by the storage treatment up to 40% in Port-Sudan, 20% in Khartoum-Sudan and 15% in UK. The result clearly demonstrated that the temperature and humidity are the crucial factors to induce the natural maturation process in *acacia* gums. Statistical analysis (linear regression) suggested statistically significant models and equations to predict and explain the variations in the physiochemical and functional properties based on the environmental factors, picking set and age of the tree.

Abbreviations

AG	Arabinogalactan
AGP	Arabinogalactan-Protein complex
ANOVA	Analysis of Variance
B. C.	Before Christ
BSA	Bovine Serum Albumin
BSG	Big Sifting Gum
CSG	Clean and Sifted Gum
C. A. S	Chemical Abstract Service
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GAB	Gum Arabic Board
GAC	Gum Arabic Company
GFC	Gel Filtration Chromatography
GP	Glycoprotein
GPC	Gel Permeation Chromatography
GPI	Glycoslyphosphatidylinstol
HD	Hashab Dust
HLB	Hydrophile-Lipophile Balance
HPS	Hand Pick Selected
HPSEC	High Performance Size Exclusion Chromatography
HYP	Hydroxyproline
INS	International Numbering System
JECFA	Joint Expert Committee for Food Additives
KG	Kibbled Gum
LIB	Lithium Ion Batteries

LLS	Laser Light Scattering
MALLS	Multi Angle Laser Light Scattering
MCT	Medium chain triglyceride
MPG	Mechanical Powder Gum
M_v	Viscosity average molecular weight
M_w	Weight average molecular weight
N.M.R	Nuclear Magnetic Resonance
O/W	Oil-in-Water emulsion
O/W/O	Oil-in-Water-in-Oil emulsion
RG	Radius of gyration
RI	Refractive Index
SANS	Small Angle Neutron Scattering
SBP	Systolic Blood Pressure
SCFA	Short Chain Fatty Acid
SEC	Size Exclusion Chromatography
SMA	Sudanese Metrological Authority
SNFC	Sudanese National Forestry Corporation
SPSS	Statistical Package for the Social Science
SSG	Small Sifting Gum
TGGE	Temperature Gradient Gel Electrophoresis
UV	Ultraviolet
VIS	Visible
VMD	Volume median diameter
WHO	World Health Organization
W/O	Water-in-Oil emulsion
W/O/W	Water-in-Oil-in-Water emulsion

Contents

Chapter 1

1. General Introduction and Literature Review

1.1 Hydrocolloids

The term hydrocolloids is used, mainly in the food industry, to describe a diverse group of long chain polymers characterized by their property of forming viscous dispersion and / or gel when dispersed in water (Milani & Maleki, 2012). These materials contain a number of hydroxyl groups which increases their affinity for binding water molecules rendering them hydrophilic compounds (Saha & Bhattacharya, 2010). Furthermore, due to their ability to produce a dispersion they also exhibit the properties of a colloid. Considering these two properties, they are appropriately termed as hydrophilic colloids or hydrocolloids (Milani & Maleki, 2012). The term hydrocolloid embraces all the many polysaccharides that are extracted from plants, seaweeds and microbial sources, as well as gum derived from plants exudates, and modified biopolymers made by chemical or enzymatic treatment of starch or cellulose (Phillips & Williams, 2009). Gelatine is a water-soluble protein derived from various collagen sources such as animal skin, bones and connective tissue by partial hydrolysis process. It has the ability of forming transparent gels and therefore widely used as gelling agent and , therefore, has been accepted as member of the hydrocolloid family (Phillips & Williams, 2009). However, other food proteins, such as casein and gluten, are traditionally not classified as hydrocolloids. Hydrocolloids are widespread in nature and include a range of polysaccharides and proteins which originate from different sources (see Table 1-1) such as plant (trees, seeds, tuber and pulses), animal (milk, meat and animal by-product) and some microorganisms (bacteria, fungi and yeast). Nowadays hydrocolloids have a wide array of functional

properties and are widely used in a variety of industrial sectors, particularly in the food processing to perform a number of functions including thickening and gelling aqueous solutions, stabilising foams, emulsions and dispersion, inhibiting ice and sugar crystal formation, and controlled release of flavours (Itthivadhanapong, Jantathai, & Schleining, 2016; Kaur, Shevkani, Singh et al., 2015; Phillips & Williams, 2009; Ramírez, Uresti, Velazquez et al., 2011). Much progress has been recently made in the applications of hydrocolloids in the field of coating and adhesives in food, as they serve as inhibitors of moisture, gas aroma and lipid migration. Hydrocolloids are unique substances and have a profound impact on food properties when used at level ranging from a few parts per million (carrageenan in heat-treated dairy products) to high levels (Gum arabic or Gelatine in Jelly confectionary) (Kralova & Sjöblom, 2009; Milani & Maleki, 2012) . The basic reason behind the application of hydrocolloids in food processing is their ability to modify the rheology of the food system. This includes two basic properties of food systems; that are flow behaviour (viscosity) and mechanical solid property (texture) (Milani & Maleki, 2012). Traditionally hydrocolloids are associated with thickening and gelation behaviour, however, due to their interfacial properties they also influence the properties of dispersion. Hence, surface active hydrocolloids may act as emulsifiers and emulsion stabilisers through adsorption of protective layers at the oil-water interface (Dickinson, 2003), and has recently attracted attention because of the physiological effect as dietary fibres, which known as non-hydrolysis materials in small intestine.

The sourcing, manufacturing and trading of hydrocolloids constitute global business where production plants can be found all over the world. The price of a

specific hydrocolloid depends widely on the availability and quality of the materials which vary from season to season depending on climate conditions during growth or harvesting and subsequent processing (Phillips & Williams, 2009).

Table 1-1. Hydrocolloids origin and sources.

Origin	Sources	Examples
Plant	Cell wall	Cellulose and derivatives, Hemicelluloses, Arabinoglactan & Pectins
	Exudates mucilage	Gum arabic, Tragacanth, Karaya and Ghatti
	seeds Gums	Guar, Locust bean, Tara and Tanarinal Inulin
	Roots	
Seaweed	Macocystis Pyrifera	Alginates
	Chondrrus Crispus, Euchema Cottoni, Euchema Spinosum	Carrageenans
	Cracilaria Gelidium	Agar
Microbial	Bacterium Xanthomonas campestris	Xanthan Gum
	Fungus *Aureobasidium* specifically A. Pullulans	Pullulan
	Sphingomonas elodea	Gellan
Animal		Chitin and Chitosan
	Animal connective tissue Collagen	Gelatine

1.2 Polysaccharides

Polysaccharides are the most abundant and diverse class of organic compounds occurring in nature. They are also the most of versatile materials available and, therefore, used widely in the development of new products ranging from foods, nutraceuticals, pharmaceuticals, textiles, paper, and biodegradable packing materials (Dickinson, 2010; Moreira, Cassani, Martin-Belloso et al., 2015). Polysaccharides are complex carbohydrate polymers made up of many monosaccharides joined together by glycosides bonds, through the elimination of a water molecule, and vary widely in terms of the composition and linkage. They tend to be amorphous in water and have no sweet taste. When all the monosaccharides in polysaccharides are the same type, the polysaccharide is called a hemoploysaccharide, but when more than one different type of monosaccharide is present, they are called heteroploysaccharide. Polysaccharides have a general formula $C_n(H_2O)_{n-1}$ when n is usually a large number between 200 - 2500, considering that the repeating units in the polymer back bone are often six carbon monosaccharides or more. A few number of polysaccharides are linear, while the majority are branched; the properties of the polysaccharides as other all polymers do not only depend on the chemical composition alone, polysaccharides can differ from each other's also in molecular weight, molecular weight-distribution, branching, crosslinking and stereo-regularity. Each of these factors has influence on the properties of polysaccharides in solution form and in bulk form. However, most of the properties are dependent on the molecular weight (Braun, H. Cherdron, M. Rehahn et al., 2004) . For example, for the solubility, the higher molecular it is, the less soluble it is, the polar groups in macromolecule enhance solubility and

short chain branches increase solubility. For the viscosity, the solution viscosity increase with the molecular weight, the branched polysaccharides show lower solution viscosities than the linear ones, even if they have the same molecular weight (Braun et al., 2004). Most of the polysaccharides are heteroploysaccharide comprising different carbohydrate. The hydroxyl groups that predominates in the polysaccharides are sometimes partially derivatized by esterification and present as acetate, sulphate or phosphate or are etherified (Stephen, M., Phillips et al., 2006) . The polysaccharides of economic importance are mainly derived from the plant kingdom (Aspinall, 1970) . They are widely distributed in the plants tissues where they play a vital role and function as; structural materials (e.g. cellulose, xylans) and as protective substances. Some plant cell wall polysaccharides are elicitors of plants antibiotics (phytoalexin) (Arman & Qader, 2012). Also, in plant cell wall, fragments of pectin polysaccharides induce synthesis of protein. Arabinoxylans have been postulated to inhibit intercellular ice formation, thus ensuring winter survival of cereals (Cui, 2005) .

Table 1-2. Sources and structures of polysaccharides.
(LAPASIN & PRICL, 1995; RINAUDO, AUZELY, & MAZEAU, 2004)

Polysaccharides	Main sources	Molecular Structure
Modified starch	Corn Kernel	Cross-linked starch molecule; some C-6 oxidized; aceteate
Carboxymethyl cellulose	Cotton cellulose	HO_2-CCH_2 groups at 0-6 of linear (1-4)-β-D-glucan
Carrageenans	Red seaweeds (*Gracilaria*,*Gigartina*)	Sulfated D-Gal, unit of (1-3)-β-D-galactan & (1-4)-3,6-anhydro-α-D-Gal
Agars	Red seaweeds (*Gelidium*)	Anhydrousugar units
Gum arabic	Stem exudate of *A.senegal*	Acidic L-Ara, (1-3) and (1-6)-β-D-Gal, highly branched with peripheral L-Rahp attached to D-GlcA. Minor component glycoprotein
Gum tragacanth	Astrsgalus	Modified acidic arabinogalactan, and modified pectin
Pectins	Citrus, apple and other fruits	Linear and branched (1-4)-α-D-galacturonan; chain include (1-2)-L-Rha*p*, and branches D-Gal*p*, L-Ara*f*, D-Xyl*p*, D-GlcA
Xanthan Gum	Xanthomonas compestris	Cellulosic structure, D-ManA and GlcA.

Alginates	Brown seaweed (Macrocystis, Laminaria and Ecklonia)	Linear (1-4)-β-D mannuronan & -α-D-guluronan.
Inulin	Chicory, Jerusalem artichokes, Onions	Fructose polymer, chain terminating by glucosyl, linked by β(1,2) $C_{6n}\ H_{10n+2}\ O_{5n+1}$

1.3 Introduction to Gum arabic

1.3.1 Background

Gum arabic is one of the oldest and most important industrial used gums. Its use can be traced back to the third millennium B.C. During the time of the ancient Egyptians, Gum arabic was used as an adhesive agent to make flaxen wrapping for embalming mummies (Caius, Radha, & Bombay, 1939), also they used gum in mineral paints when making hieroglyphs. During the 18th century Gum arabic trade was dominated by the Turkish Empire which gave rise to the name Turkey gum (Verbeken, Dierckx, & Dewettinck, 2003). However, *acacia* gum acquired the name Gum arabic due to the fact that it has been shipped to Europe through Arabian ports.

1.3.2 Definition

Gum arabic is a naturally occurring exudate from *Acacia* trees. Defined by the Food and Agriculture Organization, (FAO)/ World Health Organization (WHO) and, Joint Expert Committee for Food Additives (JECFA) as: dried exudates from the stems and branches of *A. senegal* (L,) Willdenow or closely related species to the family of *Leguminosae* (JECFA-FAO, 1999). Gum arabic is also defined as a heteroploysaccharide because it contains different carbohydrates such as arabinose, galactose, rhamnose and glucuronic acid. It also described as hetropolymolecular, because it has a variation in monomers composition and/or in the linking and branching (Anderson, Stoddart, & Hirst, 1966). The name Gum arabic is also used to denominate gum produced from other *Acacia* species, but the gum exudates from *A. senegal* trees is the major source of Gum arabic.

1.4 Regulatory aspect of gum arabic

1.4.1 Chemical and physiochemical specifications

E 414 EC*: Acacia* Gum Synonyms Gum arabic

Acacia gum is a dried exudation obtained from the stems and branches of natural strain of *A*. *senegal* (L) Willdenow or closely related species of *Acacia* family *Leguminosae*.

INS 414 JECFA / WHO:

Gum arabic is a dried exudation obtained from the stems and branches of *A*. *senegal* (L) Willdenow or *Acacia seyal*.

All regulatory definitions of Gum arabic recognize that the Gum arabic of commerce originates from more than one species of *Acacia*. According to the Food Chemical Codex, the US Pharmacopoeia (US Pharmacopoeia, 1985), the British Pharmacopoeia (British pharmacopoeia, 1993) and the Official Journal of the French Republic, gum arabic is defined as the dried gummy exudation obtained from the stems and branches of *A*. *senegal* (L) Willdenow or of the related species of *Acacia* family *leguminosae*. The European Pharmacopoeia refers *A*. *senegal* and the other species of *Acacia* of African origin, while the US Food and Drug Administration (FDA) defined as the gum originating from various species of the genius *Acacia* family *leguminosae*.

Gum arabic has been evaluated by Joint Expert Committee for Food Additives (JECFA) of FAO and WHO on several occasions between 1970 and 1998. The main discussion has been the introduction of *A*. *seyal* into the specification of gum arabic. The most significant change in the specification of Gum arabic related to its optical activity. At first, the specification of the optical rotation was defined as a 10% solution is levorotatory, it was softened in 1978, was

eliminated in 1986 (JECFA-FAO, 1999) and was introduced again in 1990 (FAO/WHO, 1990). However, *A*. *seyal* is dextrorotatory, therefore the limit for the specific optical rotation had excluded it from the specification in 1995 (FAO/WHO, 1995). Moreover, the limits for the nitrogen content which introduced to the specification of gum arabic in 1990 was also removed, since *A*. *seyal* contains less nitrogen than *A*. *senegal*. The acceptance of *A*. *seyal* as a closely related species was acknowledged at the 49th JECFA meeting in 1997 (JECFA-FAO, 1997). This specification was prepared at the 51st JECFA in 1998 and was published in (JECFA-FAO, 1999). The JECFA specification for identification and purity is given in Table 1-3.

Table 1-3. Test specified for the identification of Gum arabic. (JECFA-FAO, 1997, 1999).

Synonyms	Gum arabic (*A. senegal*), Gum arabic (*A. seyal*), *Acacia* Gum; arabic gum; INS No. 414
Definition	Gum arabic is a dried exudate obtained from the stems and branches of *A. senegal* (L.) Willdenow or *A. seyal* (fam. *Leguminosae*). Gum arabic consists mainly of high-molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid. Items of commerce may contain extraneous materials such as sand and pieces of bark, which must be removed before use in food.
C.A.S. number	9000-01-5
Description	Gum arabic (*A. senegal*) is a pale white to orange-brown solid, which breaks with a glassy fracture. The best grades are in the form of whole, spheroidal tears of varying size with a matt surface texture. When ground, the pieces are paler and have a glassy appearance. Gum arabic (*A. seyal*) is more brittle than the hard tears of Gum arabic (*A. senegal*). Gum arabic is also available commercially in the form of white to yellowish-white flakes, granules, powder, roller dried, or spray-dried material.
IDENTIFICATION	
Test	Specification
Solubility	1gram dissolves in 2ml of water; insoluble in ethanol.
Gum constituents	Proceed as directed under gum constituents identification using the following as reference standards: arabinose, galactose, rhamnose, galacturonic acid, glucuronic acid and xylose. Arabinose, galactose, rhamnose and glucuronic acid should be

	present. Additional spots corresponding to manose, xylose and galaturonic acid should be absent.
Optical rotation	Gum from *A.senegal* : aqueous solution are levorotatory Gum from *A.seyal* : aqueous solution are dextrorotatory Test a solution of 10% of sample (dry basis) in 100 ml of distil water (if necessary, previously filtered through a No 42 paper or 0.8 μm Millipore filter) using a 200-mm tube
PURITY	
Loss on drying	Not more than 15% (105° c, 5hr) for granular and more than 10% (105 °c,4hr) for spray dried Ungrounded samples should be powdered to pass through a No 40 sieve and mixed well before weighing
Total ash	Not more than 4%
Acid – insoluble matter	Not more than 1%
Starch or dextrin	Boil a 1gm in 50 solution of the sample, cool and add a few drops of Iodine T.S no bluish or reddish colour should produce
Tannin-bearing gums	To 10 ml of a 1 in 50 solution of the sample, add about 0.1 ml of ferric chloride TS no blackish colouration or blackish precipitate should be formed
Microbiological criteria	Salmonella Spp.: Negative per test E. coli: Negative in 1g
Lead	Not more than 2mg/ kg. Prepare a sample solution as directed for organic compounds in the Limit Test and determine by atomic absorption spectroscopy

1.4.2 Gum arabic as dietary fibre

The concept of the dietary fibre first emerged in the 1950s (Hipsley, 1953), and has evolved from the original physiological-botanical description as being the remnants of plant components that are resistant the hydrolysis by human alimentary enzymes. Thereafter the concept of dietary fibre was extended to include all indigestible plant polysaccharides and some chemical materials. They were included because they have the functional properties of the dietary fibre such as water holding capacity, swelling capacity, cation exchange capacity and emulsifying activity and emulsion stability. Additionally, they are resistant to the digestion process in human small intestine and complete or partial fermentation in the large intestine. (Mora, Contreras, Aguilar et al., 2013; Trowell, Southgate, Wolever et al., 1976). This broadened definition includes cellulose, hemicellulose, lignin, gums, modified cellulose, mucilages, oligosaccharide and pectin, and associated minor substances such as waxes and suberin (Phillips, Ogasawara, & Ushida, 2008). This definition gained extensive consensus and it has been considered as the gold standard definition. However the broadening of the definition to include the non-digestible oligosaccharides and resistant starch as dietary fibre, together with the recognised value of intrinsic intact dietary fibre from plant tissue and the question associated with both soluble and insoluble fibre, cause of delaying in progress to reached a universal agreement in dietary fibre definition over the past decades and has led to the realisation that the definition of the dietary fibre needed to be reviewed (Phillips et al., 2008). Because of this uncertainty in the dietary fibre definition, some products were not regulatory approved as dietary fibre despite the fact that they have similar behaviour to the dietary fibre, one of the famous of these

products is Gum arabic. Huge debate and dispute unfolded in different Codex Alimentarius sessions regard the definition of dietary fibre, there was no agreement reached in dietary fibre definition for ten years (FAO/WHO, 2001, 2003, 2004, 2005). However, the Codex Alimentarius session (32) (FAO/WHO, 2009) agreed in the following compromise definition: Dietary fibre mean carbohydrate polymer with ten or more monomeric units which are not hydrolysed by the endogenous enzymes in the small intestine of human and belong to the following categories:

- Edible carbohydrate polymers naturally accruing in the food as consumed.
- Carbohydrate polymers which have been obtained from food raw material by physiological, enzyme or chemical means and which have been shown to have physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.
- Synthetic carbohydrate polymers which have been shown to have physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

It is obvious from this definition that most, if not all, current food hydrocolloids could be considered to be dietary fibres (Phillips et al., 2008).

The most well-known method for calculating of the total fibre content of food ingredients and food products has been the enzymatic-gravimetric (McCleary, 2003; McCleary, DeVries, Rader et al., 2012), method of Association of Official Analytical Chemist (AOAC 985.29). This method used enzymatic digestion to remove non-fibre components and then weight the residue. However, over the recent decades it has become obvious that a variety of methods need to be implemented to measure the dietary fibre content for different food ingredients

(Elleuch, Bedigian, Roiseux et al., 2011). The fibre content in Gum arabic (shown in Table 1-4) varies between 80% - 90% and because of the low viscosity value of Gum arabic which might make it more suitable for use in beverage and food formation at higher inclusion levels (Viebke, Al-Assaf, & Phillips, 2014) . The usage of Gum arabic as dietary fibre in some European countries like the UK and France was approved and well accepted. There are off course implied health benefits when using Gum arabic as dietary fibre, either alone or in combination with other food ingredients (Phillips et al., 2008).

Table 1-4. Typical fibre content of some hydrocolloids and their usage limit in food application. (Viebke et al., 2014).

Hydrocolloids	Soluble fibre %	Usage limits (%)	Viscosity
Agar	85	1	High
Carrageenan	80-90	1.5	High
Gum Arabic	85	50	Low
Guar	80-85	1.5	High
Locust been	80-95	1.5	High
Pectin	80-95	2	High
Starches	60	2	High
Inulin	90	15	Low
Xanthan	80-95	1	High

1.5 Structure of *A. senegal*

Most of the work that has been reported on the structure and properties of Gum arabic dealt with *A. senegal*. Early work carried out by Anderson and co-workers (Anderson et al., 1966) on *A. senegal* gum using Smith degradation procedures indicated that the gum was highly branched polysaccharide consisting of a main chain of β-1,3-linked galactose residues as core or backbone, with extensive branched unit 1,6- linked, both the main and side chains were shown to consist of galactropyranose, arabinopyranose, arabinofuranose, rhamnopyranose, glucuropyranosyl uronic acid and 4-O-methyl glucuropyranosyl uronic acid, the latter two mostly as end units. This work was subsequently reassessed and a revised structure shown in Figure 1.1 was proposed (Street & Anderson, 1983).

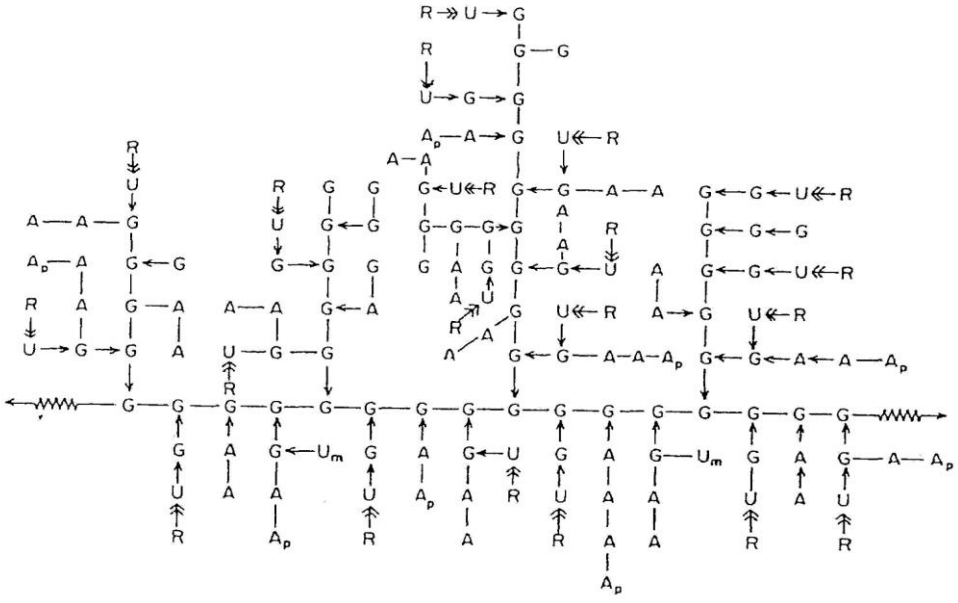


Figure - 1.1. Proposed carbohydrate structure of *A.senegal* (Street and Anderson, 1983). A= arabinose: G= galactose: R= rhamnose: U= glucuronic acid.

Subsequent study using Smith degradation and $C^{13}\ N.M.R$ proposed that the galactan core consisted of 13 units of β-(1, 3) –glucuropyranosyl residues, having two branch points giving single repeating subunits of molecular mass of 8000 Da and proposed a structural model for *A. senegal* as shown in Figure 1.2 (Stephen & Churms, 1995).

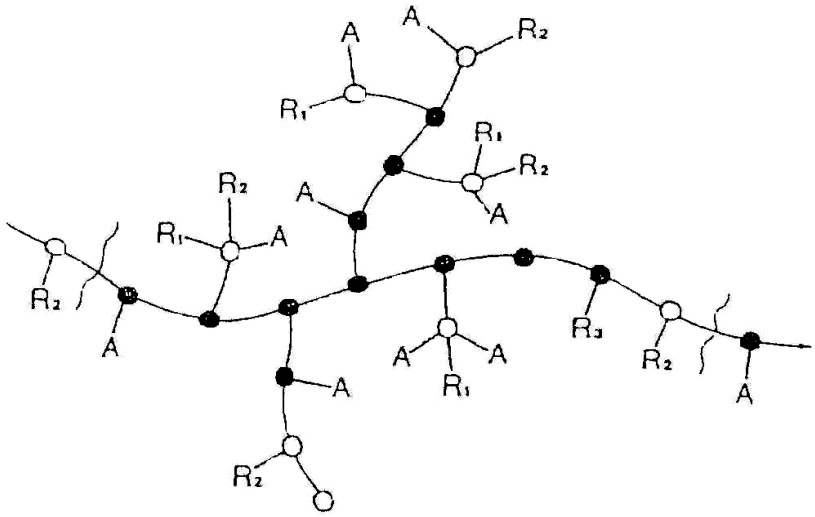


Figure - 1.2. Proposed structure of polysaccharide of *A.senegal* by (Stephen & Churms, 1995). A= arabinosyl; ● = 3 linked galactoses (galactose attached); o= 6-linked galactose (galactose or glucuronic acid attached or end group) R1= rhamnose- glucuronic acid; R2= galactose- arabinose, R3= arabinose- 3-arabinose.

Using more recently available techniques such as methylation, gas chromatography-mass spectroscopy and NMR (H1 & C13) spectral analysis another amendment to the classical structure of Gum arabic was proposed (Nie, Cui, Wang et al., 2012). They confirmed that Gum arabic (*A.senegal*) is a highly-branched polysaccharide with the backbone composed of 1,3-linked

galactopyransyl residue, substituted at O-2, O-6 or O-4 positions. The terminal sugar residues are 59.9 % of the total sugar. The residues of 2,3,6- β-D galactopyransyl, 3,4 -galactopyransyl and 3,4,6- galactopyransyl at O-2 and O-4 positions were not identified in previous studies.

Gum arabic also contains small proportion of proteinaceous material with hydroxyproline, proline and serine being the dominant amino acids. Akiyama et al (1984) were the first to show that the gum proteinaceous material is around (2% w/w) and covalently attached to the carbohydrate and demonstrate the presence of hydroxyproline-oligoarabinose and serine-carbohydrate linkage (Akiyama, Eda, & Kato, 1984). They also pointed out that the gum has been previously shown to precipitate with Yariv antigen and therefore suggested that it was kind of Arabinogalactan-protein complex (AGP); which commonly occur at the cell surface in plants and have a role in plant growth and development. Using gel permeation chromatography (GPC) technique coupled with UV detector at 214 nm Gum arabic molecular mass distribution was reported (Vandevelde & Fenyo, 1985). The resulting chromatograms illustrated four peaks, indicating that the gum contained fractions of varying molecular mass such as low molecular mass fraction containing very little proteinaceous material and a protein-rich high molecular mass AGP complex fraction which made up less than 30% of the total gum mass. Further studies carried out using enzymatic degradation by treating the gum solution with proteolytic enzyme and monitored the molecular mass distribution before and after treatment (Connolly, Fenyo, & Vandevelde, 1987). They found that the higher molecule mass components were degraded to a single component of lower molecule mass, with no change in optical rotation and equivalent weight. In subsequent study (Duvallet, Fenyo, &

Vandevelde, 1989) also reported that the molecular weight of gum decreased from 7.2 x 10^5 Da to 1.8 x 10^5 Da after being subjected to pronase treatment. Therefore both studies of (Connolly et al., 1987; Duvallet et al., 1989) suggested that their results were consistent with the '' wattle blossom '' model for AGP complex structure proposed by (Fincher, Stone, & AE, 1983) as shown in Figure 1.3 where the carbohydrate units are linked to a common polypeptide chain.

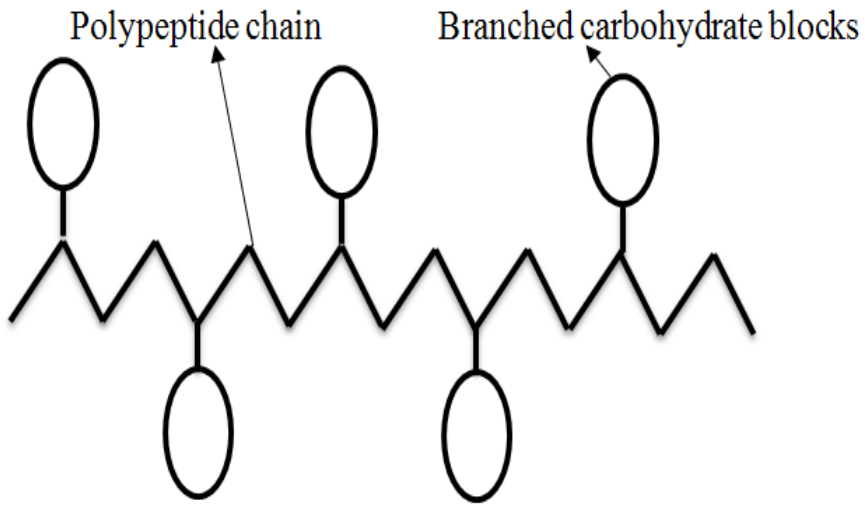


Figure - 1.3. The Wattle blossom model of the Arabinogalactan–protein (Fincher, 1983).

Connolly et al, (1987) calculated the molecular weight of the carbohydrate blocks to be of the order of 2 x 10^5 Da (Connolly et al., 1987) (Randall, Phillips, & Williams, 1988; R. C Randall, G O Phillips, & P A Williams, 1989) reported that fractionation of *A. senegal* gum by hydrophobic chromatography revealed three distinct fractions: a high molecular mass Arabinogalactan-protein (AGP) consisted of 10% of total gum mass with 12% protein and molecular mass of 1.45 x 10^6 Da and hydrodynamic radius of ~23 nm, a low molecular weight

fraction Arabinogalactan (AG) which makes up 80% of the total gum mass and has only 0.35% protein with molecular mass ~ 2 x 10^5 and the smallest fraction glycoprotein (GP) contain ~1% of total mass with highest protein content ~ 50 wt %. Afterwards Osman et al (1993), also fractionated the gum using hydrophobic affinity chromatography and found that the molecular weight of the AGP component decreased to ~ 2 x 10^5 Da on treatment with proteolytic enzyme (Osman, Menzies, Williams et al., 1993). Their results were consistent with above finding (Connolly et al, 1987, Duvallet et a*l*., 1989) and the '' wattle blossom '' model for AGP complex. Gel permeation chromatography (GPC) couple with refractive index (RI), UV and laser light scattering detectors demonstrated that the molecular mass of *A*. *senegal* fractions varies from 2 x 10^6 to 2 x 10^5 (Williams & Langdon, 1995). Subsequently study by Idris et al, (1998) investigated the molecular weights of 8 authenticated *A*. *senegal* samples by using the GPC technique. They determined radius of gyration and also calculated the hydrodynamic radius by using photon correlation spectroscopy (PCS) of all of the molecular species. They reported that the hydrodynamic radius to be small relative to their molecular mass. Therefore, their study concluded that there was evidence of a highly branched globular structure consistent with the ' wattle blossom' model proposed for arabinogalactan protein complexes (Idris, Williams, & Phillips, 1998) .

Another study for *A*. *senegal* structure carried by Qi et al, (1991), by separation the *A*. *senegal* gum using chromatography, they pointed out the two major fractions (GAGP) possesses a highly repetitive, hydroxyproline rich, polypeptide backbone. They postulated that small (~ 11residue) repetitive peptide theme each with three hydroxyproline (Hyp)-arabinose attachment sites

and a single Hyp-arabinogalactan polysaccharide attachment site. They demonstrated that GAGP molecules of *A. senegal* were rod-like ~ nm by electron microscopy and described the structure of GAGP with twisted hairy rope model shown in Figure 1.4 (Qi, Fung, & Lamport, 1991). Additionally, Goodrum et al, proposed the new model of GAGP. They reported that a family of closely related peptides confirmed the presence of a repetitive 19 residue consensus theme which was about twice the size anticipated by Qi et al.(Goodrum, Leykam, & Kieliszewski, 2000).

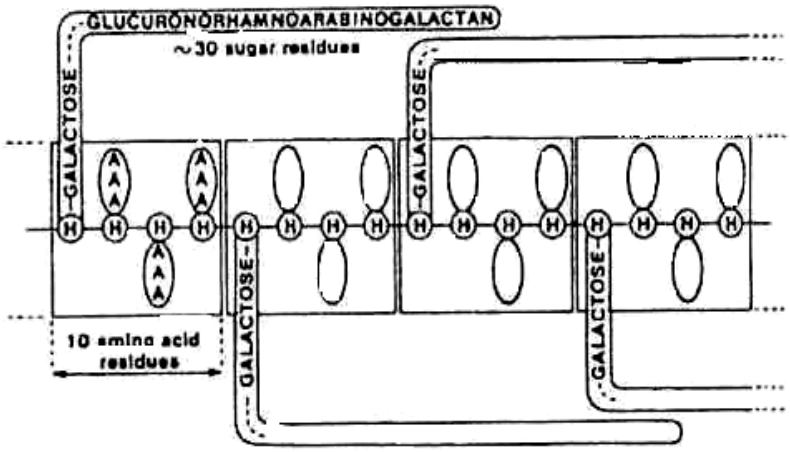


Figure - 1.4. Twisted hairy rope" model of gum arabic glycoprotein.

Further study of *A. senegal* structure carried by Sanchez et al, (2008). They concentrated on (AG) fraction and using high performance size exclusion chromatography–multi angle laser light scattering (HPSEC-MALLS), and small angle neutron scattering (SANS) coupled to Ab initio calculation and various microscopic techniques they provided the first thin disk model for the (AG). It's a disk- like morphology with a diameter of 20 nm and a thickness below 2 nm as shown in Figure 1.5 (Sanchez, Schmitt, Kolodziejczyk et al., 2008).

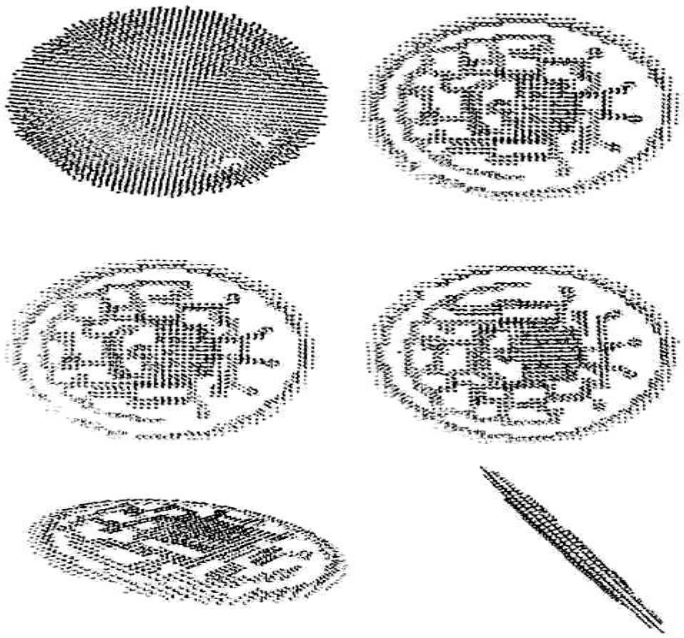


Figure - 1.5. Different views of the most probable DAM of F1 as Thin Oblate Ellipsoid model.

Additional study, on the structure of *A. senegal* was carried by Mahendran et al, (2008). They monitored the composition and physiochemical properties before and after enzyme treatment and various alkaline hydrolysis. GPC-MALLS result of molecular weight and radius of gyration indicated that the macromolecules have compact structure and the AGP fraction consist of carbohydrate blocks of ~ 4.5 x 10^4 Da linked to polypeptide chain through both *O*. serine and *O*. hydroxyproline residues consistent with the Wattle blossom model. Using deglycosylated analysis revealed the presence of the two putative core proteins of ~ 3 x 10^4 and ~ 5 x 10^3 Da, which correspond to protein of approximately 250 and 45 amino acid in length. Therefore, a revised model structure of AGP was proposed and shown in Figure 1.6 (Mahendran, Williams, Phillips et al., 2008).

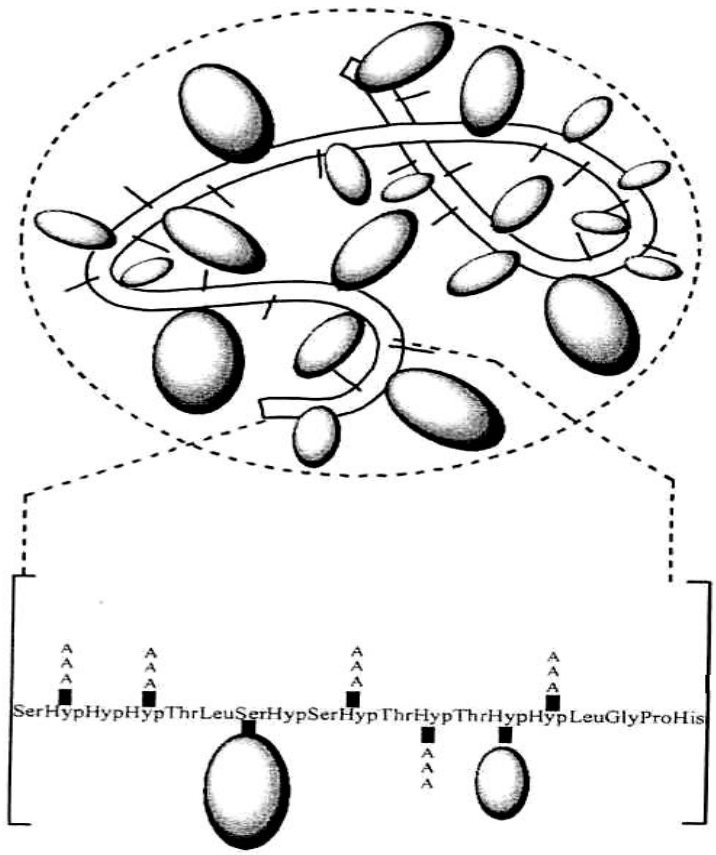


Figure - 1.6. Schematic illustration of the structure of Gum arabic Arabino-galactan protein complex.

More studies by (Youl, Basic, & Oxley, 1998) have shown that AGPs from cultured cells of tobacco (*Nicotiana alata*) and pear (*pyrus communis*) and of rose (*Rosa sp*) (Svetec, Yadav, & Nothnagel, 1999) are synthesized with glycosylphosphatidylinositol (GPI) lipid anchor. Furthermore, the deduced amino acid sequences of AGP genes from other plant species, including *Arabidopsis thaliana*, also contain putative sites for the attachment of GPI anchors (Schultz, Johnson, Currie et al., 2000; Sherrier, Prime, & Dupree, 1999). Therefore, Yadav et al (2006) speculated that glycosylphosphatidylinositol (GPI) lipid anchor were present in the AGP fraction (Yadav, Johnston, Hicks et al., 2006).

1.6 Physiochemical properties

The physicochemical properties of the polymers are generally determined by their size, shape and structure. The configuration of the polymer is partly dependent on the main chain and partly on the various sides groups (Laurier, 2005). In case of Gum arabic, the properties and characteristics vary significantly depending on different factors, such as, geographical, age of the trees, climatic conditions and soil environment (Idris et al., 1998). as well as also the place of exudation on the tree (Islam, Phillip, Sljivo et al., 1997).

1.6.1 Moisture content

There have been numerous studies dealing with the moisture content of Gum arabic (Al-assaf, Katayama, Phillips et al., 2003; Al-assaf & Phillip, 2006; John, Phillips, & Williams, 2012; Karamalla, Siddig. N, & Osman. M., 1998). It is now widely accepted that Gum arabic has high moisture content immediately after picking (up to 15%) but subsequently this value is decreased following drying. Typical values reported in the literature range from 10-15 % with an average of ~ 12% for gum nodules and a lower value of ~ 10% for broken (kibbled) gum.

1.6.2 Optical rotation

The specific optical rotation was considered as the most important criterion of purity and identity of Gum arabic. In the revised specifications (FAO/WHO, 1990) *A. senegal* gum specific rotation falls within the range between -26 to -34. Early studies reported that the average of specific optical rotation for *A. senegal* gum from different age of trees was -30.0 and the range is -27 to -32 (Anderson et al., 1966) and -29 to -34.4.

In a comprehensive study (K. Karamalla et al., 1998) dealing with gum samples from 1960 – 1995, further investigated the effect of location, age of tree and rainfall. They reported an average of -27.3 for areas with rainfall <400mm and -29.3 for > 400mm. Also, reported different average value of -33.1, -31.8 and -32.6 respectively for first, second and third picking.

1.6.3 Viscosity

Gum arabic is a highly water soluble gum and can be dissolved up to 50% in water with relatively lower viscosity compared to other gums. This unique property is due to its highly compact and branched structure (Glicksman, Sand, & Whistler, 1973). The intrinsic viscosity of freshly collected gum varies from 14 to 60 cm^3/g and the mean viscosity changes with the age of the crop and season (Duvallet, Fenyo, & Vandevelde, 1993). In a study on 1500 commercial and authenticated gum arabic samples of *A*. *senegal* var *senegal* originated from Sudan the mean average value at 16.44 cm^3/g with a range from 14-73 cm^3/g was reported (Karamalla, Siddig, & Osman, 1998). They observed that the viscosity of *A*. *senegal* gum dose not only vary with anticipated factors such as gum concentration, temperature, pH and cationic composition but also with less obvious factors such as mesh size, storage period and even the method of preparation of samples.

1.6.5 Nitrogen content

Various studies have reported the nitrogen content of Gum arabic samples used in the respective investigation. A conversion factor of 6.5 was then used to calculate the total protein content and a wide range of values were reported in various studies. For example, 2.2 -2.8% (Anderson & Smith, 1967), 2.1 – 2.5%

(Osman et al., 1993), 1.5 – 2.6% (Idris et al., 1998), 2.2% (Al-assaf et al., 2003) and 1.8 – 2.2%.(Elmanan, Al-Assaf, Phillips et al., 2008).

1.6.6 Molecular mass and molecular mass distribution

The molecular weight of *A. senegal* show wide variation which can mainly be attributed to the method used for determination and heterogeneity of samples, (Glicksman et al., 1973).

There are several different methods and techniques to calculate and measure the molecular weight of substances, generally the compatibility, of using specific technique depend on materials and the targeting molecular weight. The most commonly used techniques are Osmometry, ultra-centrifugation, molecule sieve chromatography, gel filtration, fractional conservation, intrinsic viscosity measurements and light scattering. However, the most confident results were afforded by light scattering measurements of the molecule weights on fractions obtained by fractional precipitation (Anderon & Rahman, 1967). Using light scattering method the reported weight average molecular weight (M_w) for *A. senegal* gum to be around 3.84 x 10^5 (Anderson & Dea, 1969). Using stearic exclusion chromatography (SEC) the reported value was 5.6 x 10^5 for the molecule weight (M_w) for whole gum (Churms, Merrifield, & Stephen, 1983). The molecular weight of Gum arabic and fractions obtained by hydrophobic fractionation were determined by light scattering (R. C Randall et al., 1989). They reported a value of 4.6 x 10^5 for the whole gums and 2.79 x 10^5 for fraction (1) arabinogalactan (AG), 1.45 x 10^6 for fraction (2) Arabinogalactan – protein complex (AGP) and ~ 2.8 x10^5 for fraction (3) glycoprotein (GP). Using light scattering (LS) technique and gel permeation chromatography (GPC) (Osman, Menzies, Williams et al., 1994; Osman et al., 1993). They reported a value of

$2.0 – 3.0 \times 10^5$, $1.0 – 2.0 \times 10^6$ and 2.0×10^5 for the three fractions AG, AGP and GP respectively. Studying the molecular ~~e~~ weight of *A. senegal* gums from trees of different age and location by using GPC coupled with LS (Idris et al., 1998). They reported value of $2.0 – 7.9 \times 10^5$ for the whole gum. The highest value of M_w was obtained from samples of trees 15 years old and they also reported a value of, $1- 4 \times 10^6$, $1.5 – 3.0 \times 10^5$ and 1.8×10^5 for AGP, AG and GP respectively. Another study reported a range of $5.3 \times 10^5 – 8.8 \times 10^5$ for the whole gum and $1.9 \times 10^6 – 3.75 \times 10^6$ for the high molecular weight fraction AGP (Al-assaf et al., 2003). Using also GPC coupled with LS (Al-Assaf, Phillips, Aoki et al., 2007). The reported a value of 8.34×10^5 for whole gum, value of 3.06×10^6 for AGP and a molecular weight and value of 4.73×10^5 for AG + GP fractions. Further studies using GPC and LS (Elmanan et al., 2008) , They reported value a range from $3.3 \times 10^5 – 4.8 \times 10^5$ For the whole Gum arabic.

1.7 Emulsification properties

An emulsion is colloidal dispersion system comprises a collection of small particles dispersed in a continuous liquid phase of different composition (Laurier, 2005). The dispersed phase is sometimes referred to as the internal (disperse) phase and the continuous phase as the external phase (Laurier, 2005). Emulsions have been defined as an intimate mixture of two immiscible liquids, in which one liquid phase is dispersed throughout the other in form of small discrete droplets (Glicksman et al., 1973). In most emulsions one of the liquids is aqueous while the other is hydrocarbon based and referred to as the oil phase. There are two types of simple emulsions which are readily distinguished in principle, depending upon which kind of liquid forms the continuous phase:

- Oil-in-Water emulsion (O/W) for oil droplet dispersed in water. This is the most versatile emulsions and its properties can be controlled by varying both the emulsifier used and the components present in the aqueous phase. e.g. Milk liquid, Mayonnaise and vanishing cream etc.
- Water-in-Oil emulsion (W/O) for water droplet dispersed in oil. e.g. Butter and cold cream

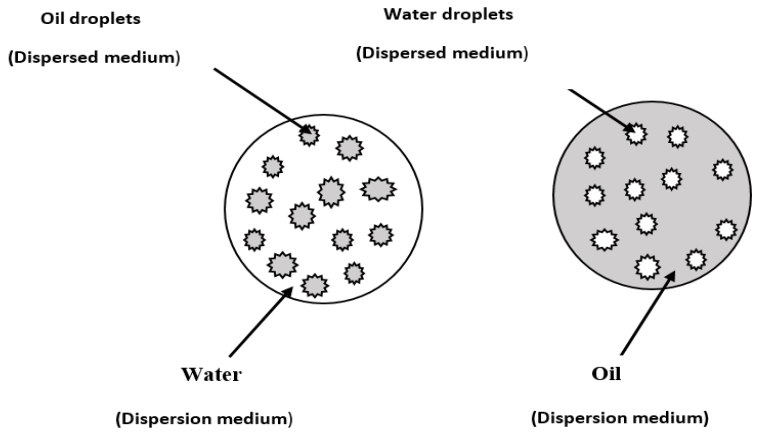


Figure - 1.7. Two simplest types of emulsions, oil-in-water (O/W) and water-in-oil (W/O).

However, practically the situation of emulsion preparation is not always so simple and one may encounter double emulsion:

- Oil-in-water-in oil emulsion (O/W/O) for oil droplets dispersed in aqueous droplets that in turn dispersed in continuous oil phase. The most common double emulsions and they are used for some specific applications such as drug (Benichou, Aserin, & Garti, 2004).
- Water-in-oil-in water emulsion (W/O/W) for water droplets dispersed in oil droplets that in turn dispersed in continuous aqueous phase. In double emulsion,

the droplet size has been greatly exaggerated and the droplets can be very large (Laurier, 2005).

Hydrophile-Lipophile Balance (HLB) is probably the most useful approach to predicting the type of emulsion that will be stabilized by given surfactant. The LHB concept was first introduced by Griffin (Griffin, 1949) to describe the balance of the size and strength of the hydrophilic and lipophilic groups on an emulsifier molecule. In the case of non-ionic emulsifier, the probability of the emulsion can be predicted by HLB value according to the Griffin formula define HLB by equation 1-1.

$$\text{HLB} = 20Mh/M \qquad [1\text{-}1]$$

Where Mh is the molecular mass of hydrophilic group and M is the molecular mass of the whole surfactant molecule. For the LHB the dimensionless scale ranges from 0–20 for non-ionic surfactants; a low HLB (<9) refers to a lipophilic surfactant (oil soluble) and a high HLB (>11) to a hydrophilic (water soluble) surfactant. Most ionic surfactants have HLB values greater than 20. In general, water-in-oil (W/O) emulsifiers exhibit HLB values in the range 3–8 while oil-in water (O/W) emulsifiers have HLB values of about 8–18 (Laurier, 2005).

Emulsion could possibly have different range of appearances rather than the familiar classical milky appearance, depending upon the droplets size and the difference in refractive indexes between the phases, therefore emulsions can be transparent, if either the refractive index of each phase is the same, or alternatively if the dispersed phase is made up of droplets that are sufficiently small compared with the wavelength of the illuminating light.

Oil and water cannot coexist comfortably due to the surface energy in the oil – water interface, therefore it necessary to reduce the interface tension by

introducing an emulsifier. In the formation of emulsion system, here it is quite important to distinguish between two types of ingredient; the emulsifying agent (emulsifier) and the stabilizer (Dickinson & Galazka, 1992). An emulsifier is a single chemical or mixture of components requiring substantial surface activity at the oil-water interface and has the ability to facilitate the formation and stabilization of fine droplets during and after the emulsification process. Most of the emulsifiers are amphiphilic molecules which have polar (Hydrophilic) and nonpolar (Hydrophobic) sides in the same molecule (Dickinson & Galazka, 1992; Evans, Ratcliffe, & Williams, 2013). The distinguishing features of an emulsifier are listed below:

(i) It must be surface-active which means has the capacity to lower the tension at the oil-water interface where the lower interfacial tension, the greater the extent to which droplets can be broken up during intense shearing or turbulent flow (Walstra & Smulders, 1998).

(ii) Ability to retain small droplets during the emulsification.

(iii) Ability to quickly adsorb at the new oil-water interface and create a transient stabilizing layer.

There are two broad classes of emulsifying agents used in the field of food processing; small molecules surfactants which are distinctly amphiphilic molecules and macro-molecular emulsifier (Dickinson, 2003). The term bioemulsifer or biosurfactant are also commonly used for the emulsifier in the field of biotechnology and applied microbiology (Navon-Venezia, Zosim, Gottlieb et al., 1995), but they are rarely used in the field of food industry, where the term emulsifier is confined to low-molecular weight amphiphilic form.

In comparison a stabilizer is a single chemical component or mixture of components conferring long term emulsion stability by increasing the viscosity of the continuous phase of emulsion and they enhance the stability by retarding the movement of the droplets; they are normally biopolymer-proteins or polysaccharides (Dickinson, 2003). Small molecular surfactants are not so effective in conferring long term emulsion stability. The main stabilizing action of the polysaccharide is via viscosity modification or gelation in the aqueous continuous phase. The effect of the stabilizer on the emulsion could be noticed in different terms such as, no visible change in the size distribution of the droplets, no change of the state of aggregation or no change in their spatial arrangement within the sample container over the time-scale.

Most of the polymers could be an effective stabilizer if they exhibited specific characteristics such as: surface activity, capacity to lower the tension at the oil-water interface, strong adsorption which implies that the polymer must has substantial degree of hydrophobic character to keep it permanently anchored to the interface, complete surface coverage and this implies that there is sufficient polymer present to fully saturate the surface, formation of thick steric stabilizing layer and formation of a charge stabilizing layer and this indicate the presence of charge groups on the polymer that contribute to the net repulsive electrostatic interaction between particle surface shown in Figure 1-8. This characteristic is only important only if the thick steric stabilizing layer not sufficiently thick (Dickinson, 2003).

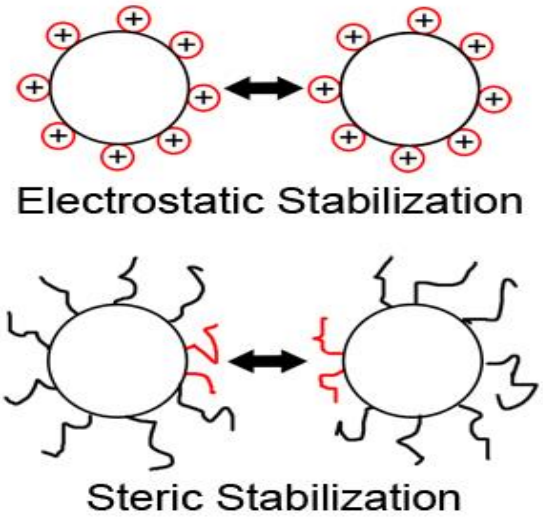


Figure - 1.8. Illustration of electrostatic and steric stabilization.

Emulsion stability is the term used to describe the ability of an emulsion to resist changes in it is properties over time .However, this stability could be affected by so many factors such as; the droplet preparation factor which includes, (homogenization method, type of the emulsifier, oil and water ratio, emulsification equipment, concentration of emulsifier, temperature, pH and viscosity), nature of interfacial adsorbed layer (interactions and competition of adsorbed species), nature of continuous phase (rheology, ionic environment, solvent quality and unabsorbed polymers) and nature of dispersed oil phase (solid/liquid content and solubility in continuous phase). Emulsions are not thermodynamically stable; rather they possess some degree of kinetic stability and must consider such things as what degree of change and over what time scale. An emulsion may become unstable through different physical mechanisms such as creaming or sedimentation which usually happened due to the density difference between the dispersed and continuous phases in which droplet move upward or downward, mostly the densities of oils are lower than that of water,

and so it tend to accumulate at the top of an emulsion and water at the bottom thus droplets in oil-in-water emulsion tend to cause creaming, where those in water-in-oil emulsion tend to cause sedimentation); aggregation this happened when two or more droplets clump together possibly touching at some points and with virtually no change in total surface area; flocculation which occurs when two or more droplets come together to form an aggregate in which the droplet retain their individual integrity; and coalescence is the process when two or more droplets merge together to form a single layer droplet (shown in Figure 1.9) (Laurier, 2005).

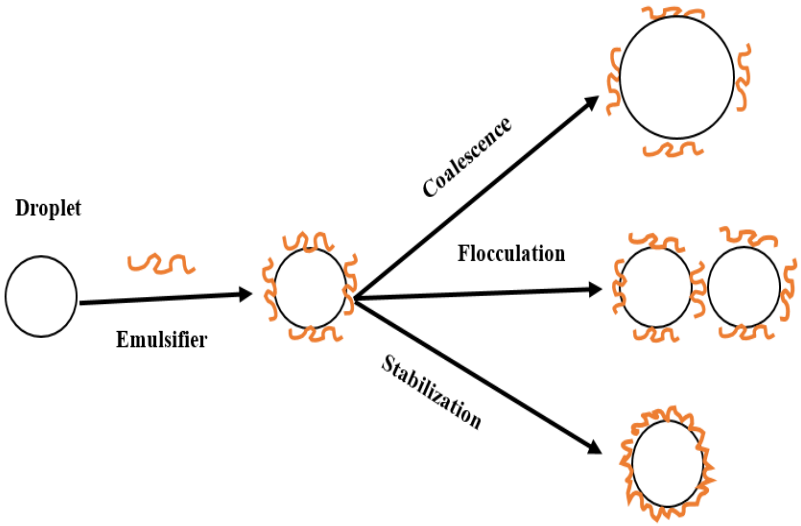


Figure - 1.9. Physical mechanisms for stability of emulsions

Generally, most the hydrocolloids (polysaccharides) have a little surface activity at oil-water interface and they do not normally adsorb at the hydrophobic surface of interface layer, therefore, they are not useful as emulsifying agent. However, there are some exception among them such as; gelatine, Gum arabic, modified starch or cellulose, guar gum and pectin (Dickinson, 2003). Gum arabic

(*A*. *senegal* and *A*. *seyal*) is the most commonly used emulsifier; in food, pharmaceutical and printing industries; precisely *A*. *senegal* species is major specie that used in commercial emulsification for the production of beverages and flavours concentrates (Thevenet, 1988).

Studying the manners of various hydrocolloids (including Gum arabic) as an emulsifier agent revealed that the emulsifying capability of the polysaccharides can be elucidated in terms of complexion and contamination with the small fraction of surface active protein, this is certainly true of Gum arabic. The AGP fraction is mostly responsible for the emulsifying properties, the hydrophilic carbohydrate blocks are linked to the protein chain that strongly adsorbs at the oil– water (O/W) interface promoting emulsion stability. Removal of the protein by treatment with proteolytic enzyme greatly reduces the emulsification properties (Randall et al., 1988). Recently Yadav et al, reported that the present of Glycosylphosphatidylinositol (GPI) lipid in the AGP fraction and that these also make a contribution to the emulsification properties (Yadav et al., 2006). Further work however, is required to confirm these. The increase of AGP percentage in emulsion is helped to achieve more uniform homogeneity of the oil droplet population (Castellani, Guibert, Al-Assaf et al., 2010), and that has reduced the proportion of large droplets (McClements, 1999) which consequently induce emulsion stabilization according to Stoke's law (Equation 1.2) where the upward movement of oil droplets in an emulsion, causes creaming destabilisation is proportional to the square of their radius.

$$V = \frac{2r^2(d_2 - d_1)g}{9\mu} \qquad [1.2]$$

Where V is velocity of separation (or rate of creaming) (cm/sec), r is droplet radius (cm), d_2 is density of continuous phase, d_1 is density of disperse phase (g/cm^3), g is acceleration of gravity and μ is viscosity of the continuous phase (g/cm.sec).

Recent studies (Castellani, Guibert, et al., 2010) confirmed that the proportion of the different Gum arabic components in particularly AGP to induce different emulsifying behaviour, this is in good agreement with the finding of (Dickinson, Murray , Stainsby et al., 1988) which suggested an influence of the distribution between the low molecular weight fraction (AG and GP) and high molecular weight (AGP) and also with (Randall et al., 1988) who first identified the predominant role of the AGP fraction. However, there does not appear to be direct correlation between nitrogen content and emulsifying effectiveness.

The hydrophobic protein component of the (AGP) assertively endure the protein-polysaccharide moiety at the interface and the protruding hydrophilic carbohydrate blocks which are attached to the protein chain provide a strong steric hindrance to prevent the flocculation and coalescence of the emulsion droplets (Buffo, Reineccius, & Oehlert, 2001). However, there are so many other factors that affect the Gum arabic emulsion stability such as; minerals which could decrease the emulsion stability presumably due to an electrostatic screening effect, pH also affect the emulsion stability where emulsions are less stable at pH (2.5) than at higher pH levels (4.5-5.5), moreover both pasteurization and demineralization favoured stability of the emulsion, most likely by promoting protein unfolding and eliminating the screening effect (Dickinson, Galazka, & Anderson, 1991).

Gum arabic is not strictly standardized in it is functional properties, therefore, uneven performances of the gum as emulsifier and stabilizer may arise among different shipment because of dissimilar fractionally related to species, varieties, geographical location, individual growing season, post-harvesting processing and age of tree (Jurasek, Kosik, & Phillips, 1993). Consequently, the variability in the emulsifying properties of the gum samples, from different acacia species, is dependent not only on their total protein content, but also on the distribution of the protein/ peptide between the low and high molecular weight fractions and on the molecular mass of the protein/ peptide for adsorption. It therefore important to choose consistently the same species and growing region to reduce at least this natural variability (Buffo et al., 2001).

1.8 Objectives

Gum arabic is produced in the hottest and most arid parts in Africa. Its journey to the international market starts with the local farmer, local trader, local trader companies, processing companies which are mostly in Europe and US and up to the end user (mostly in a convenient powder form) for different applications such as food, pharmaceutical and other industries. These various steps of the gum journey are known to vary greatly and could have critical effects on its quality and performance.

This study charts the way to gain some knowledge about the natural and external factors that influence the quality of gum. Better understanding of these factors, therefore, will undoubtedly contribute to achieving material of good and consistent quality.

The belt of *Acacia* trees in Sudan cover many regions which differs in many factors such as environmental factors (rainfall, soil type, temperatures, humidity), age of the trees, time of picking and number of picks. All those factors contribute to the natural built in variations on the quality of *acacia* gums. The first study to investigate these factors was carried out on twelve samples of *A. senegal* from three different districts of Sudan and thirteen different single nodules from *A. senegal* trees of different ages. They reported that the molecular weight for *A. senegal* gum varied widely from sample to sample (Anderson, Dea, Karamalla et al., 1968). They showed that the viscosity is dependent on the age of the trees and also noted the regional variations. More characterization study to investigate the influence of location and age of trees on *Acacia* gums physiochemical properties was subsequently reported on 8 samples of *A. senegal*, 4 samples from one plantation in the western and eastern region,

from trees of different age. They reported that the monosaccharide composition, protein and amino acid contents were not affected by age or location of tree. Their major finding is the correlation of molecular weight and age of tree where the highest molecular weight in samples from 15 years old from the western region only but no correlation in samples from eastern region. In this above mentioned study the samples were collected from one pick during one season. (Idris et al., 1998). A comprehensive study provided analytical data for 1500 authentic and commercial *A*. *senegal var*. *senegal* gum samples, collected from different locations, age of tree and storage conditions. Their findings indicated a wide variation in intrinsic viscosity and viscosity average molecular weight. Consequently, they could not recommend these parameters as suitable indices for quality control (K. A. Karamalla et al., 1998). Recently, (M. E Ballal, E. A El Siddig, M. A Elfadl et al., 2005), investigated the influence of the environmental factors on the yield of the *A*. *senegal* gums in one plantation in the western region of Sudan. They reported that the quantity of produced gums was found to be positively correlated with tapping intensity, rainfall, and the minimum and maximum temperature at tapping time and negatively correlated with tapping time and the minimum and maximum temperature during collection. Their conclusion shows a significant relationship between environmental factors and production.

In this study, 168 authenticated samples were collected from the western and the eastern regions in Sudan. The samples differ in the environmental factors, age of the tree, location, rainfall, picking time and collections number. All the samples were collected during 2008-2009 from six plantations namely Demokeya, Nabag, Twobon, Khor Donia, Bozey and Trees belt, which covered

most of the gum belt in Sudan. The experimental work was designed to determine the physicochemical and chemical properties. These include moisture content, optical rotation, intrinsic viscosity, ash content, molecular weight, protein content. In addition, this study will involve the evaluation of the functional properties according to the recent methods used to characterise gum arabic by using gel permeation Chromatography (GPC) coupled with three different detectors, used by (Al-Assaf et al., 2007; Al-Assaf, Phillips, & Williams, 2005; Elmanan et al., 2008). Moreover, the study will evaluate the influence of the all above mentioned factors on the gum functional properties such as evaluation of emulsion performance and stability. The objective of the work is also to provide a definite answer whether the location, collection and age of tree affect the physiochemical and functional properties or not. Secondly, to investigate the effect of storage conditions in three different locations (hot and dry, hot and humid and cold and humid) influence the quality of *A*. *senegal* gums (Gum arabic) over 5 years from the date of collection.

Chapter 2

2. Gum arabic indigenous knowledge, Production, Processing, Marketing and Applications

2.1 Origin of the Gum

The production of the gum or the gummosis is widely encountered throughout the plant kingdom. Gum formation in plants is induced by environmental stressors such as infection, insect attack, flooding, and mechanical or chemical injury. All of these factors stimulate gum exudation, believed to act via ethylene produced in plant tissues (Miyamoto, Kotake, Boncela et al., 2015; Skrzypek, Miyamoto, Saniewski et al., 2005). The basic reason of the gummosis or formation of gum by *Acacia* trees is still not fully understood but many theories have been formulated to explain these phenomena. For example, some thought that they are a product of normal plant metabolism or due to pathological condition (Smith & Montgomery, 1959). Others believed that the formation of gum is a result of microbial infection of the injured tree and the plant synthesises the gum exudates in order to seal off the infected section and prevent further invasion of the tissue. Additionally, it has also been attributed to fungus growth on the plant with the liberation of fungal enzyme which then proceeds to synthesize the complex polysaccharide gums. It is, however, widely accepted by many that Gum arabic is produced by trees only when they are in unhealthy conditions. In Sudan, the largest producer, many producing farmers believe that gum yield is directly correlated with abundance of certain beetle *nubeculosus* locally known as ‘’ Garraha’’ there is no clear evidence so far to grantee this correlation (Hassan, 1999). The anatomical site of gummosis was examined by comparison of the carbohydrate composition of the tissues from the inner bark,

the cambial zone and the xylem of gum production branch with the corresponding tissues of a branch which do not produce gum (Joseleau & Ullmann, 1990). Water-soluble polysaccharides and hemicelluloses were extracted from anatomical zone for both branches. The results showed that acidic polysaccharides were plentiful in the aqueous extracted from inner bark and cambial zone of the gum producing branch and were absent from the non-gummiferous branch. Natural sugar and uronic acid analysis showed that the acidic polysaccharides extracted from inner bark and cambial zone had the same composition as the gum produced by the same branch. The identity of the gum was further demonstrated by methylation analysis and C^{13} NMR spectroscopy. The same biochemical showed that the presence of gum in the cambial zone is not restricted to the site of gum exudation and the gum existed on both site of the wound on the branch even beyond the limits of tapping point. It is widely accepted now that gummosis is promoted when the tree is subjected to stress conditions such as heat, drought and insect attack, but no one so far has proposed a universally accepted explanation.

2.2 The ecological importance and the socio-economic factors associated with Gum arabic production

Almost all the produced gum in Sudan mainly comes from wild natural habitat. However, there are a few reserved plantations owned by the government and the private sector. Most of the farmers in the gum belt in Sudan have no formal education and practice the traditional agriculture as their basic occupation. The producers of Gum arabic are typically peasant farmers picking gum exudates as secondary or complementary source of revenue. Typically, none of the farmers get any kind of financial assistance from the government or from commercial

banks (Hayward, 2004). Collection of wild non-timber forest products, such as Gum arabic, contributes to improving the livelihoods of communities through their direct consumption or marketing.

2.3 Geographical location of *Acacia* trees

About 1350 species of *Acacia* trees are distributed in tropical and subtropical areas and arid zones areas of Africa, India, Australia, and Central America, but few are commercially important. The *Acacia* trees are concentred in Australia (955 species), with also high numbers in Americas (185 species), Africa (144 species) and Asia (89 species) (Al-Assaf et al., 2007). The important production areas are Sudan, French West Africa and several neighbouring countries. In Africa, the *Acacia* trees grows widely across sub Saharan from Mauritania, Senegal and Mali in the west, through Burkina Faso, Niger, northern parts of Nigeria and Chad to Sudan, Eritrea, Ethiopia and Somalia in the east, and northern parts of Uganda and Kenya, they form the so called "gum belt.

2.3.1 Geographical location of *Acacia* trees in Sudan

The phrase gum belt is used to denote a zone of approximately 200,000 square miles, which is now divided between two countries after the spilt of the Sudan recently in 2009 to two countries North Sudan and South. In North Sudan, the gum belt extends across central and South between latitude (11^{o}and 12.5^{o}) north, which accounts for roughly one-fifth of the country's total area. The belt accommodates around a fifth of the population of the Sudan (IIED & IES, 1990) and is comprised of nine states divided into western and eastern regions. The western regions are the traditional source of gum arabic in Sudan which includes west Darfur, South Darfur, North Darfur, North Kordofan, West Kordofan and

South Kordofan. Eastern regions are the new plantations and include Senar, Blue Nile and Gadareef as shown in Figure 2.1.

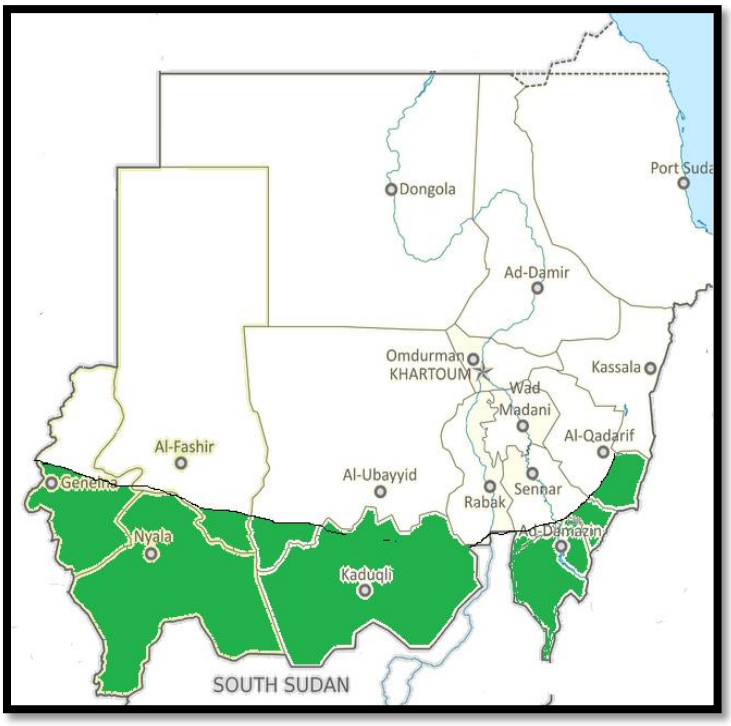


Figure - 2.1. Gum arabic belt in North Sudan.

In general, there is a clear environmental variation between the two regions such as soil type, rainfall, temperatures and humidity also in the skills, technical know-how and tools of tapping. For example, farmers in the western region employ a new tool of tapping which called (*Sunki*) shown in Figure 2.2. The application of this tool, unlike the previously used axe, is considered to be less intrusive and helps to improve the quality of the produced gum collection and handling of the final product (see also section 2.6).

Figure - 2.2. Tapping tools in North Sudan.

According to a survey carried by the International Institute of Environment and Development, and the Institute of Environmental Studies (IIED & IES, 1990) there are some evidences to suggest that the belt has moved southwards and no *Acacia* trees exist north of latitude 13^{o} 45 North. Vegetation maps based on satellite image carried by Ministry of Enviroment Development in Sudan (MOED) also depict the southward shift of the belt (MOED., 2003).

In South Sudan, the gum belt is considered as the natural extension to the gum belt across North and central South Sudan between latitude 9°and11°N. The gum belt in South Sudan is composed of six regions namely Upper Nile, Unity, Jonglei, Warrap, North Bahr el Ghazal and Western Bahr el Ghazal as shown in Figure 2.3.

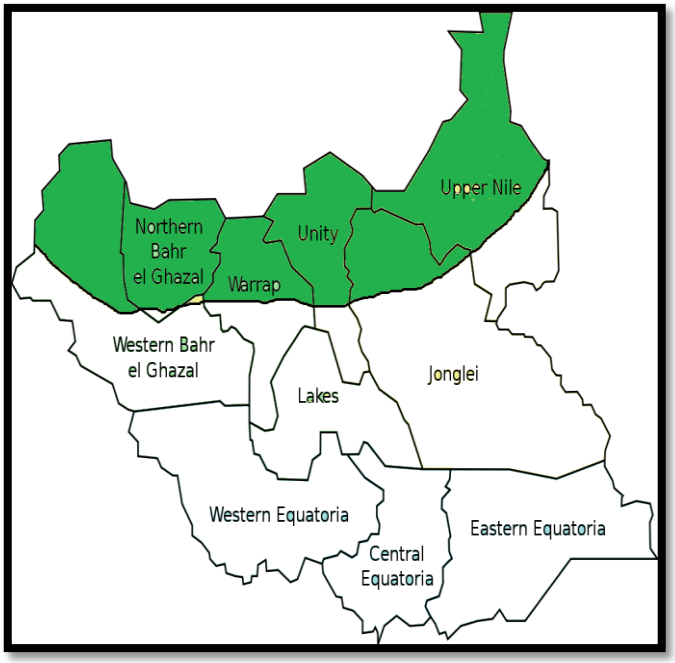


Figure - 2.3. Gum arabic belt in South Sudan.

2.4 Climatic and Soil types in Sudan

2.4.1 Climate and rainfall

Two climatic regions are present in Sudan whereby an arid zone towards the North to tropical wet-and-dry in the far southwest with a large range of climate sub-regions forming an extensive savannah in the south with rainfall from 400-1000 mm to semi desert in the North. Temperatures do not vary greatly with the season at any location but the most significant climatic variables are rainfall and the length of the dry season (Metz, 1991). Rainfall is the most vary climatic factors in the gum belt combined with soil type. The average annual rainfall determines the distribution of *A*. *senegal* and thus the location of the gum belt. According to the environmental data from Sudanese Metrology Authority (SMA) in the west, the annual rainfall ranges from 250- 600 mm and in the east from 400 – 1000 mm (SMA., 2013).

2.4.2 Soil

Sudan soil can be divided geographically into three categories. These are (i) the sandy soil (so-called *Goz* sands) of the northern and west central, (ii) the clay soil known as cracking soil which is widespread in central and southeast and (iii) the laterite soil of the south (Metz, 1991). Basically, the gum belt comprises two types of soil, sandy in the west region and clay in the east region. In the west region sand soil, can be found either pure or mixed with clay and so-called locally (*Gardout*). Large areas of north and south Kordofan, southern Darfur and patches of the White Nile state are covered by sandy soil, in the form of sandy Pedi plain soils, the different form of sandy soil, sand sheets and sand dunes grouped under the name of (*Goz*). Within this group are numerous flat patches and depressions of alluvial soils deposits ranging from loam to clay. Depth, high permeability and relatively high availability of water during the dry season make these soils the best substratum for *Acacia* species growing as shown in Figure 2.4.

Dark cracking clay soil, often referred to as (black cotton soils) are the flat uniform plains of Gadareef, Blue Nile, Seenar, upper Nile and some parts of south Kordofan. These plains are characterized by deep cracks when dry, low permeability and relatively low water availability during the dry seasons as shown in Figure 2.4.

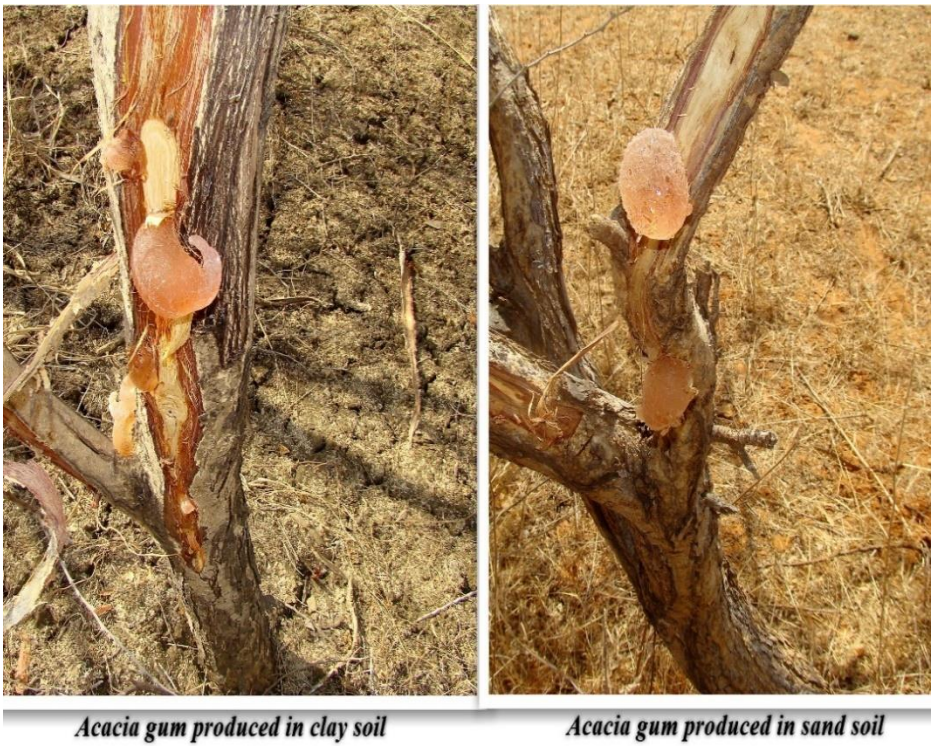


Figure - 2.4. Different type of soil in Sudan gum belt.

2.5 Classification of most known *Acacia* Species in Sudan

Acacia tree belongs to the *Acacieae* within the sub-family *Mimosoideae* of the family *Leguminosae* (the *Pea* Family). The classification of *Acacia* genus which have gradually mature over time, first with Bentham (Bentham, 1842, 1864, 1875) which predominate for about one century and subsequently slightly modified by Vassal (Vassal, 1972), Pedley (Pedley, 1978, 1986, 1987a, 1987b, 1989) and Maslin (Maslin, Miller, & Seigler, 2003) are shown in Table 2-1.

According to taxonomic classification for *Acacia* genus by Bentham, which is the one that most widely used, classified *Acacia* into series; the most known once are series 4 (*Gummifera)*, also called *A*. *seyal* complex, which include *A*. *nilotica* (syn-*A*. *arabica*), *A*. *nubica*, *A*. *seyal* (varieties *seyal* and *fistula*) and *A*. *tortilis*.

Series 5 (*Vulgares*, subgenus *Aculeifer*), also called *A. senegal* complex, the famous species in Sudan belong to this series are *A. Polyacantha* sub-species *complyacantha*, *A. laeta*, *A. senegal* and *A. mellifera*.

*A. senega*l and *A. seyal* remain the most commercially exploited species of the whole *acacia* resource (Al-Assaf et al., 2007). However, *A. polyacantha* export has seen an increase recently according to the report by the Gum Arabic Board in Sudan (GAB) (GAB, 2014).

2.5.1 *A. senegal*

A. senegal is a thorn trees that reaches a height of 4.5-6 meters. It is very drought resistant; thrive on dry rocky hills, in low-rainfall dry savannah, and areas where annual rainfall is 100mm – 950mm and dry period of 5-11 months (Chikami, 2001; F. John, Kennedy, et al., 2012). It is also tolerating high daily temperatures of up to $45C^{\circ}$. This hardy species survives many adverse conditions, and seems to be favoured by low rainfall and absence of frost. *A. senegal* specie has four different varieties which are; *A. senegal- variety–senegal* it recognised as a tree with flat or rounded crown and rough non-papery and non-peeling bark, *A. senegal*-variety-*Kerensis*, grown as several stemmed shrubs with lateral branches from near the base and with smooth yellowish brown peeling bark on the stem, *A. senegal* (L) Willd *var rostrate* Brenan and *senegal*- variety-*Leiorhachis* Brenan (B. Chikamai., M. Muga, & Marangu., 2012).

2.5.2 *A. Seyal*

A. seyal recognised by it is smooth stem which is covered with whitish, greenish yellow or orange-red powdery layer, bright yellow flowers in heads and the sickle-shaped dehiscent pods that are constricted between seeds. *A. seyal* species

has two varieties: *A. seyal var seyal* and *A. seyal var fistula*. *A. seyal var seyal* is characterised by a reddish or reddish brown and occasionally green back, spines are sharply pointed, more or less straight in pairs and normal; *A. seyal var fistula* is distinguished by whitish or greenish yellow bark, pairs of spines fused at the base forming pseudo-galls, often called ant-galls because they are often associated with ants (John, Phillips , & Williams 2012).

2.5.3 *A. Polyacantha*

Also, known as white thorn, it is flowering tree which recognised by growing up to 25 meters tall and by reddish brown bark which is useful for tanning. *A. polyacantha* is the one of the species in series 5 (Vulgares), sub-series gerontogeae spiciflorae of Benthman's classification of the genus *acacia*. *A. Polyacantha* has two varieties: *A. polyacantha var complyacantha* and *A. polyacantha var polyacantha* (Osman, Osman, Hassan et al., 2012) .

Table. 2-1 Most known *Acacias* species in Sudan and their local name

Acacia scientific Name	Local Name in Sudan
A. senegal	Hashab
A. seyal	Talah
A. polyacantha	Kakamut or Abou-Seenena
A. laeta	Shubahi
A. melifera	Kitr
A. nilotica	Sunat
A. seberiana	Kuk
A. nubica	Al-seedier
A. tortilies	Al-seyal

2.6 Gum arabic tapping, collecting, marketing and exporting

Production of Gum arabic is a long process composed of different and various steps. One of the most important steps in Gum arabic production is the pre-harvest activities, which usually done by individual farmers, and includes husbandry, tapping (system, tools, timing and location), which mainly depended on the age of the gardens. The corner stone of the pre-harvest activities, is the system of agriculturally shifting cultivation, which known to be called the bush fallow system. It is the system that based and developed from the farmer's indigenous knowledge and their understanding to the ecosystem in order to sustain production of all crops including gums (Elrayah, Osman, Al-Assaf, & Ali, 2012). In the bush, fallow cultivation system, farmers tend to fell old gum garden when they are over twenty years old and leaving one or two trees as seed bearers. The land cultivated along for four to five years with different field crops such as millet (Pennisetum typhoides), sorghum (Sorghum bicolour), sesame (Sesame indicum), groundnuts (Archis hypogaea) and roselle (Hibiscus sabdariffa), the activities of farmers while tending the field crops, provide protection for the *Acacia* trees seed to germinate and resulting of increase seedling. Then farmers shift to another plot of land leaving the *Acacia* trees to dominate, and forming sequential tree agricultural crops shifting cultivation (M. E Ballal et al., 2005). However nowadays the bush fallow system in gum belt in Sudan is infrequent or non-existent and diminishing due to so many factors such as misuse of land, drought, desertification, the civil war and the spilt of Sudan into two countries. These factors also contributed to the fluctuation of gum yield and consequent instability of supply and price.

2.6.1 Tapping

Tapping is a process of manually wounding the tree whereby the bark is removed. It is an important activity carried out by farmers during the early part of the dry warm season, when the trees are just starting to shed their leaves. Currently in some areas there are two tapping seasons, especially at open *Acacia* forests. The first one during November / December and the second March / April. Previously the onset warm dry season started early during October / November but changed now due to the shift of the gum belt to the south where the rainy season is significant. However, in most areas tapping is still done once year but a number of collections are carried out from the same tapped area. The older method of tapping is by making small incision in the branches and stem of the trees by using an axe. This method has now been largely being replaced, by new technique, which utilizes a specially designed sophisticated tool called *Sunki* (see Figure 2.2). It has a metal head fixed to a long wooden handle whereby the pointed end of the head is pushed tangentially into the stem or branch to penetrate just below the bark and then pulled up to strip-off a small length of bark. Several branches are treated in similar manner at one tapping. Although gum can be tapped from the trees after 3 years but the accepted practice commonly employed is start tapping only after 5 years and (Williams & Phillip, 2000) continuous until the trees over 20^{th} years old . After five weeks of tapping the first collection of gum is made manually as partially dried. Further collections from the same trees of approximately two weeks intervals until the end February which makes four to five collections in total depending on the weather conditions and the health of the tree (Imeson, 1992). The last collection is usually open for all residence of the area regardless of the owner of the garden

and locally called (*JAGHAT*) which simply means no rule. They do this to show a kind of solidarity with the poor people in the community (GAC, 2009) . Farmers in the western region are better trained and have developed good techniques of tapping to increase the yield, improve quality, minimize or eliminate damage to forest resource or trees health risk (FAO/WHO, 1995). Their tapping is characterised as very shallow and only tap two third of the branches and leave the stem of trees. In contrast farmers in eastern regions are mostly still using axes in tapping. Additionally, there seems to be clear difference also in terms of tapping all branches and stems as well as deep tapping compared to the shallow in western regions. Figures 2.5 and 2.6 shows examples from the respective region.

Figure - 2.5. Tapping technique in the western region - very shallow and mainly concentrate on branches.

Figure - 2.6. Tapping technique in the eastern region - very deep and often on the stem of the tree.

2.6.2 Gum collecting and local marketing

The gum yield is lower in trees five to ten years old and increase to reach its peak at 15 years then decreases at 20 years (Abdel Rahman, 2001). The first collection is made usually 5 weeks after tapping but this depends on the weather conditions. The yield of collected gum is the highest in the second pick then decreases slightly in the third and sharply thereafter (M E Ballal, E A El Siddig, M A Elfadl et al., 2005). Farmers practice their own indigenous knowledge during the collection time and have developed a system to follow accordingly. For example, it has been well established that a drop-in temperature during the period collection is detrimental to the yield of gum. Therefore, the gum producer particularly in the Western region have developed a practice for starting the collecting at midday or when the weather is hot so that the gum nodule can be easily picked from the tree. The explanation for this practice stated recently, that low temperature could lead to the sealing of exudation points (IIED & IES, 1990). Dried gum picked by hand and collected in small open basket from different trees in one certain area. The collected gum is allowed to further dry before being transferred to bags, which are commonly polyethylene. It is estimated that the average annual production from *A. senegal* tree ranges from 0.5 to 0.9 kg of crude gum nodules and the estimation average of farmers' production is 650-850 Kg per annum (GAC, 2009). The collected gums, then stocked for a short period of times, usually not more than a week before delivering to the village merchants or the middle-traders. At this stage, the gum is kept either in closed polyethylene bags or by digging a hole in the ground to store it. Framers adopted this storage method to avoid losing weight during the

drying period of the gum. Usually the normal period of storage by farmers is around one week but it can be significantly prolonged depending on the price in the local market. The consequence of a longer storage can significantly affect the gum quality where agglomeration of the gum in a big sticky lump and sticking to the bag which lead to gum mould, colour change to red contamination with foreign materials such as stone, sand, grass, and mud. Most farmers are often not able to sale their gums directly in auctions market or directly to trader companies due to lack of finance capability to cover the transportation, packaging, government taxes, and the labourers cost. The middle-trader usually peddles around villages to collect the gums and in some cases the middle trader is one of the village merchants. More commonly, the middle traders deal and agree with the farmers before the tapping season giving them an advanced loan mostly by providing them with essential food commodities such as sugar, cooking oil, onion, salt, sorghum flour, tea, coffee ... etc. According to this system, the price of the gum will be agreed and decided in advance by the farmers and the traders. Locally this system called (Shial) and usually favours the middle traders. The farmers then bring their gums in small quantities in plastic bags to the village merchant or middle traders and as result of lack of weighing system a local weighing tools called (Coora) is used. This is made up of a small pot' which approximately equal to 1.5 Kg. Gums sold unclean and unsorted and it checked visually by the middle traders and immediately packed in jute bag with small punctures to allow the fresh gum to dry gradually and avoid the formation of sticky lump pieces and colour change. In some areas, particularly in the eastern region where other gum varieties such as *A. seyal* and *A. polyacantha* are present the process involves checking every bag to ensure

that the gum is not mixed. The collected gum from different farmers in different villages is then packed in 100 Kg jute bags. Some of the traders clean and sort the gum but the majority sale it as crude gum to the big trader or in the auction market to the exporting companies or any other traders.

2.6.3 Cleaning, sorting and packing (Big traders stage)

Generally, the distinguished characters of a big trader are (i) wealthy person, (ii) based in a city near the gum belt or the auction market, (iii) has the finance capability to buy and receive huge quantities of gums from middle traders and farmers and (iv) the-ability to store this gum for long period if required. In some cases, the storage period can be up to one year to reach the highest profits possible. Typically, the gum purchased by the big trader is visually inspected for some physical parameters such as the colour, size, quantity of the broken gum, impurities percentage and subsequently stored according to the origin of the gum. The reputations of the middle trader are the key factor which determines the level of inspection to determine the variety and also to avoid mixing with lower quality and price such as *A. polyacantha*. It is very difficult to distinguish the difference between *A. senegal* and *A. polyacantha*-when they are fresh but the difference can be obvious when the gum modules start to dry. The big traders are usually suppliers of one of the exporting companies. In the past, most of the received gum by the exporting companies was cleaned and sorted. Nowadays the plurality of the received gum from suppliers to the exporting companies is in form of crude gum without cleaning or sorting (GAC, 2009). Figure 2.7 shows the various stages described above.

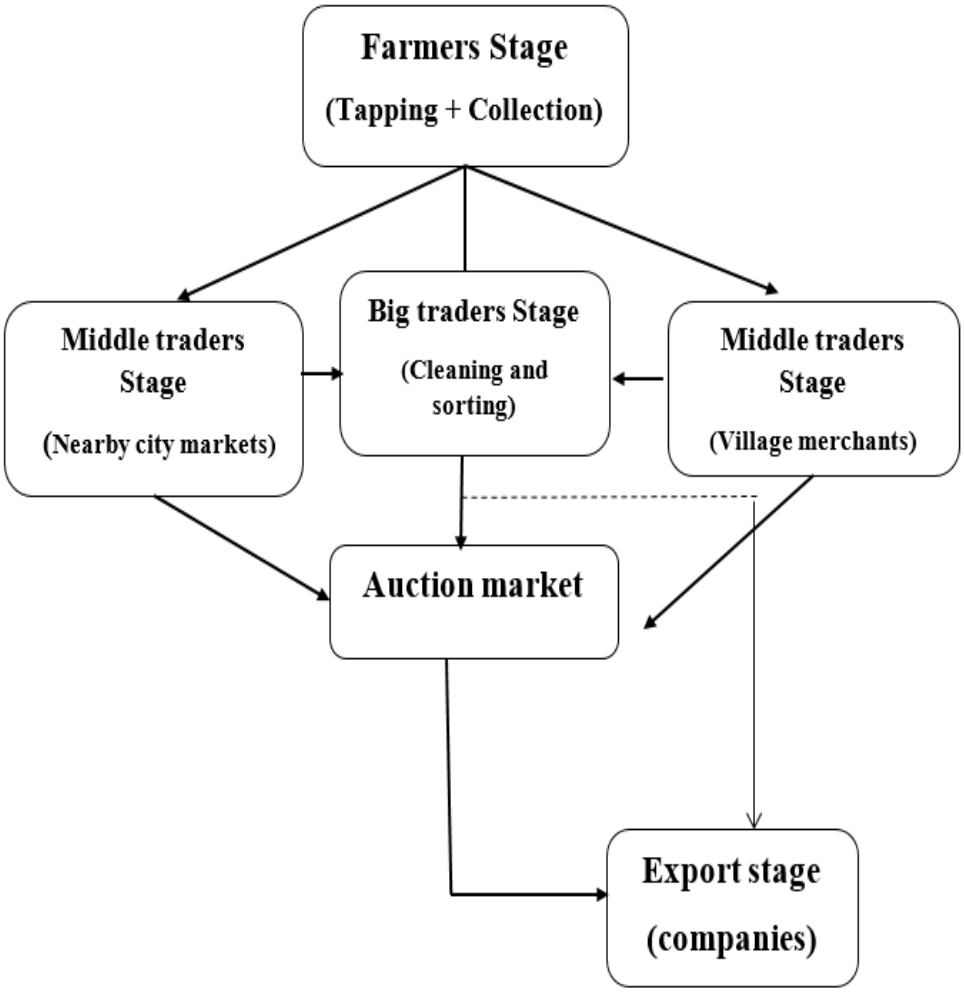


Figure - 2.7. Gum arabic production and marketing channels in Sudan.

2.6.4 Commercial grads of Gum arabic from Sudan

Cleaning and sorting traditionally done by women. They sort the gum according to the size of lumps and colour and remove foreign matters such as barks, stone and sand, mud and grass (FAO/WHO, 1995).The gum can be further processed mechanically by breaking up large lumps into smaller granules with a more uniform size distribution.

Gum arabic produced from Sudan, has two major grades, *A. senegal* (Hashab), and *A. seyal* (Talha). *A. senegal* grade also includes two sub grades, crude gums, and processed gums, and also the two sub grades had many various sub-grades.

Crude *Acacia* gum (Hashab) the most exported grade from Sudan which accounts to ~ 75 - 80 % and has many sub- grades. These include Hand Pick Selected (HPS) which is the clean large nodules mostly not broken with the lightest colour and commands the highest price. Clean and sifted (CSG), which has a colour varies from pale amber to dark amber, and it is mixed between lumps and broken gums ~ < 6.5mm. The clean grade is the standard grade used throughout the world market and is the most exported grade from Sudan. Sifting is the by-products from the manual cleaning of the HPS and CSG and contains a percentage of impurities ~ 10 % and it is also divided to two types; Big siftings (BSG), ~ > 6.5 mm < 2 mm, and Small siftings (SSG), ~ >2 mm < 0.8 mm. The final grade is Hashab Dust (HD), >0.8mm, collected after the cleaning process, containing a high percentage of impurities over than 25%. Kibbled gum or processed gum has uniform size and the gradation of kibbled gum are depending on the size formation and purity, it has two grade, Mechanical powder (MPG), it is very clean, the impurities > 0.3%, and the mesh size 125 µ or 210 µ, and commands the highest price. Kibbled gum (KG), (GAC, 1995). The various grades are shown in Figure 2.8.

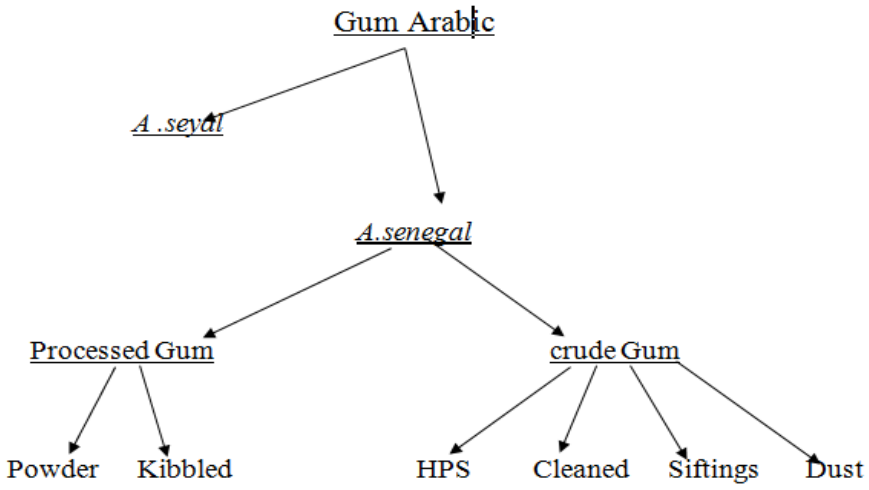


Figure - 2.8. Commercial grads of Gum arabic from Sudan.

2.6.5 Exportation and the international market of Gum arabic

A previous estimate put the value of world market for gums used as food additives at about 10 Billion US dollars, of which the two largest 'forest gum' (Gum arabic and locust bean) account for just 12% which takes no account of non-food uses of gums (FAO/WHO, 1995). The demand of Gum arabic alone is estimated between 55000 and 70000 metric tonnes annually. It has been kept up by the increase in the global consumption of drinks and confectionery and promoted by the search for quality and natural products. Because of its soluble fibre content, new applications are being developed in dietary and health foods, and a market that is booming in western countries. Locally, Gum arabic usage in producer countries is very limited small quantities in food products, in traditional pharmacopoeia as well as in manufacture of ink and paint (FAO/WHO, 1995). The big producers' countries are Sudan, Nigeria and Chad respectively and produce almost 95% of the Gum arabic exported to the world market. Fifteen other African countries produce the gum with exported volumes below 1000 tonnes a year; the most important are Ethiopia, Tanzania, Cameron and Senegal. Between 2001 and 2010, exports developed with pronounced variation between 25000 and 95000 tonnes, average of 50000 tonnes per year.

Sudan has dominated the production and trade of Gum arabic, accounting for 80-90% of the world market (Chikamai, Banks, & Anderon, 1996). In the last two decades Sudan lost a part of it is market share but still remains the highest ranking (UNCTAD, 2013). Naturally the gum belt in Sudan has been seriously evanescence because of, excessive *acacia* trees cut coupled with the severe frequently drought and desertification as well as factors such as economic or

political instability. Economically, the fluctuation of the price in local market push the farmers to seek other alternatives, which obviously led to excessive cut of *acacia* trees in favour of expansion of agricultural activities or charcoal production. Also, part of erosion of Sudan position in the world market caused by the development of the *A*. *seyal* gum, which is less expensive; Chad, the leader in this segment, saw its production increase greatly. Moreover, the new awareness of economic benefit of Gum arabic business, and the huge development in gum sector in some African countries, led to an increase of the production for those countries and therefore decrease of Sudan share globally. Politically the civil war in Sudan particularly in the gum production areas caused a massive displacement of the local farmers, and therefore the production of Gum arabic is decreased, in addition to the recent split of Sudan into two countries. All these factors together are seriously threatening the dominant position of Sudan in Gum arabic world market. In the late 1960s and begging of 1970s Sudan Gum arabic production was in excess of 60,000 tonnes per year, but in mid of 1970s and 1980s the production of the gum in Sudan is decreased sharply due to the climatic and political events. In 1973-1974, a severe drought occurred in the Sahelian gum belt, resulting in the collapse of gum production to around 20,000 tonnes per year (Imeson, 1992). Sudan production of Gum arabic was recovered to around 40,000 tonnes per year at the end of 1970s, but new drought in 1982-1986, together with political and civil conflict, brought production down to below 20,000 tonnes per year at the end of 1980s. In early 1990s, the production further decreased due to cold weather and rainfall fluctuation. At the end of the 2000s, Sudan gradually liberalised the industry and in 2009 the Gum arabic Company's (GAC) monopoly on marketing and

exporting Gum arabic was abolished and similarly the policy of minimum prices governed by the Ministry of Foreign Trade was suspended, consequently the Gum Arabic Board (GAB) was created shortly afterwards to coordinate the implementation of reforms and to support efforts to stimulate the sector, and this led to the slightly recovered in production around 30,000. However, the deterioration and the fluctuation of the production of Gum arabic in Sudan is still likely to happened at any time, due to political interfere in Gum arabic business, civil conflict and unclear business policy, which subsequently led to fluctuation in price and earn. Illustrated in Figure 2.9. (UNCTAD, 2013).

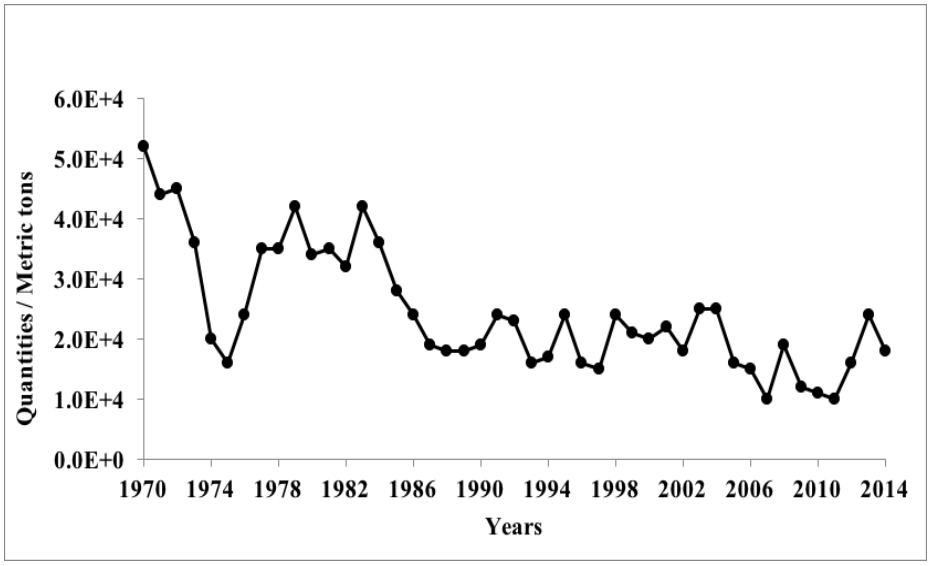


Figure - 2.9. Exported quantities in metric tons from Sudan for 45 years (GAC & GAB).

The European community is the biggest regional market for Gum arabic, and imports into it, with import averaged almost 28000 tonnes / year. France and United Kingdom are the biggest market 'Although they both re-export a large proportion of their imports'; followed by Italy and Germany, outside the

European countries, United State is the largest market followed by Japan and India. Imports increased by 55% between 2001 and 2010, with only 2009 showing a fall following the international financial and economic crisis. In 2010, 123 206 tonnes of Gum arabic were imported, of which 28% by France, 13% by the United States, 7.3% by the United Kingdom, 6.07% by India (but 18% in 2009), and 5.36% by Germany. With the exception of India, the major importers are European (France, United Kingdom, Germany, Italy, Belgium, Ireland), plus the United States and Japan. India already imported 9000 tonnes of gum in 2001 but its volumes doubled to reach almost 20,000 tonnes in 2009. Another major development was that in a few years China became the 8th global importer with an increase of 308% in volumes imported between 2001 and 2010. On a smaller scale, but with volumes increasing regularly, Brazil (+127% between 2001 and 2010), the Russian Federation (+334%), and Poland (almost fourfold) also emerged. But these countries, unlike India, primarily import processed gum from Western countries. A certain depolarisation of the Gum arabic market can be seen, which will only become more marked in the years to come with an increase in demand from emerging countries. The traditional Gum arabic markets (United States, Europe and Japan) could see their influence diminish with an increase in purchases by emerging countries directly from the African producing countries (UNCTAD, 2013). The price for both export and import for the main countries show very strong disparity between the different countries. This could be explained by the different species and quality of raw gum, the degree of processing and country power of negotiation. Export price for producing countries are very variable, but much less than those for re-export. For *A. senegal* variety *senegal*, which produced in Sudan, and well known by Kordofan gum is

the highest. For *A*. *seyal* the highest price is the variety of *fistula* which knows as white *seyal*, Chad is the reference with price slightly higher than that of Sudan and Nigeria. Although Gum arabic is unique natural products, which has versatile applications, but remains inelastic in term of the price, the principal factor which dictates the gum price is supply; where currently the demand for gum arabic is in increase worldwide. Gum arabic price shot up to reached record levels in 2004/2005 above five Thousand Dollar per tonne. Therefore the price of Gum arabic remain in the highest level since 2011, and all Symptoms of the gum market expected that price will keep to increase; and that because of weather changing due to the shift of gum belt to new production areas where the rainy season is quite long (higher and longer rainfall than average, delay in tapping the trees), insufficient stocks (nether in Sudan nor in the others African produced countries) and as well as the security condition in Sudan after the spilt into two countries (UNCTAD, 2013). The competition between synthetic alternatives and natural gums is getting harder, and cause significant decline in use of many gums, and that due to industry's general preference for new materials which are of consistent, predictable quality, and are not subjected to the vagaries of weather, plagues of locusts, insufficient funding for smallholders and economic and political stability in producing countries. In many cases, synthetic alternatives are technical superior to the natural gums. However, some natural gums are still used widely; Gum arabic is prime example, either because they have functional properties which synthetic materials cannot match or because they are available at price which makes it cost-effective. In addition in food application particularly, any change of formulation requires a change in the labelling of the end product, which very costly, and also there are marketing

advantages for the food producers if they label their products as containing natural contents rather than synthetic additives and ingredients (FAO, 1995).

2.7 Toxicity and food safety evaluation of Gum arabic

Gum arabic was firstly approved by the United State (US) food and drug administration (FDA) for use as food additives and listed as generally recognized as safe (Doi, Ichihara, Hagiwara et al., 2006; FDA, 1974). However, the safety evidence has been derived from a long history of human consumption in local production areas in Africa as food and in the treatment element in India or globally. Nevertheless, Gum arabic has been subjected to series of rigorous toxicological testing and safety evaluation such as those conducted by JECFA in 1969 (Phillips et al., 2008) then again by (FAO/WHO, 1982) which produced comprehensive toxicological monograph. It also has been reported that Gum arabic has no potential sub-chronic toxicity or carcinogenicity (Anderson, Ashby, Busuttil et al., 1982). Further assessment conducted in 1989 when new data were available and toxicological monograph accession was produced (FAO/WHO, 1990). Inclusive investigations concerning the safety of conventional preparations of Gum arabic were conducted and employed a wide range of dose level into the animals, both with or without metabolic activation. The test results revealed that no indication that modified gum *acacia* possessed neither mutagenic nor teratogenicity potential and the animals are not adversely affected by acute or sub-chronic exposure to modified gum *acacia* (FAO/WHO,1982,1993; Scmitt et al., 2008; Sheu et al., 1986).

The available evidence of the gum arabic safety so far covers the following:

- Metabolism and pharmacokinetics: based on food and caloric intake in rats and guinea pigs, at dietary levels of up to and above 10% the gum was virtually completely digested and utilised (Phillips et al., 2008).
- Acute toxicity: the acute toxicity of *acacia* gum has been evaluated in the mouse, rat, hamster and rabbit and was shown to have a very low acute toxicity. The median lethal dose (LD50) was in the range 8–18 g/kg body weight as a bolus dose, equivalent to between 500 g and 1 kg for a 60-kg adult human (Phillips et al., 2008).
- Sub-acute/sub-chronic toxicity: short-term tests showed Gum arabic to be practically devoid of toxicity at doses up to 10% of the diet in mice and rats, 15% of the diet in guinea pigs and 20% in rabbits. There were neither gross nor microscopic lesions detected but just small effects on body weight were seen was reduced in mice and rats at the highest dose levels but increased in guinea pigs and rabbits.
- Genotoxicity: these studies have been conducted in a number of bacterial and yeast strains in vitro, Drosophila and in a dominant lethal assay in rats. These tests confirmed the lack of genotoxicity of *acacia* gum.
- Chronic toxicity/carcinogenicity: long-term tests of 2 years' duration have been conducted in both rats and mice at dietary levels of up to 5%. No increase in tumour incidence was observed and there were no other signs of chronic toxicity, confirming the safety of the material on prolonged exposure (Phillips et al., 2008).
- Developmental studies: teratology studies were conducted in rats, mice, hamsters and rabbits and were found consistently negative. Some adverse maternal effects were reported in the rabbit at high dose levels of 173 or 800

mg/kg body weight but the dosing was by gavage (gastric stomach tube) as single bolus doses daily and this protocol commonly causes gastrointestinal dysfunction in rabbits. There were no dose-related changes in maternal findings, number of foetuses, foetal viability or external, visceral or skeletal variations. No terata were seen and it was concluded that Gum arabic was neither teratogenic nor carcinogenic (Welsh, Black, Graham et al., 1987).

2.8 Applications of *A. senegal*

2.8.1 Gum arabic a dietary fibre and prebiotic

The definition of the dietary fibre which agreed by codex Alimentarius in 2009 (see section 1.4.2), is well-fit with the structure and properties of Gum arabic, whereas it is edible, natural polysaccharide consist of more than 10 monomeric units, and it has been approved that gum arabic is non-digestible and resistant the hydrolysis by the endogenous enzyme in the small intestine and passed down to be fermented by intestinal bacteria to produce Short Chain Fatty Acid (SCFA), particularly propionic acid in the large intestine (Adiotomre, Eastwood, Edwards et al., 1990). Further work by (Kishimoto, Ushida, Phillips et al., 2006) showed that total production of SCFA is directly proportional with the molecular weight value of Gum arabic samples, their finding also confirmed that the sharp rise in the net production of SCFA after the initial 8 hours lag-time, and it continued at on exponential increase of concentration for 24 hours after the start of incubation, and beside the propionic acid Gum arabic produced smaller amount of butyric and valeric acid. Furthermore, in their study by using Temperature Gradient Gel Electrophoresis (TGGE) analysis during the incubation of Gum arabic shown that the responsible bacteria for Gum arabic fermentation in the large intestine is *Prevotella ruminicola*.

It has also been reported that a diet high in dietary fibre has significant impact in the health improvements (Slavin, 2003), which led to the public and industrial consciousness that dietary fibre is a substantial ingredient in a healthy diet (Brown, 2011; Gidley, 2013; Kendall, Esfahani, & Jenkins, 2010; Metha, 2009). This led to the official recommendation that a certain amount of dietary fibre be a daily part of the diet (Chawla & Patil, 2010; Redgwell & Fischer, 2005). Most recommendation for adults suggests a fibre intake about 25g/day (Gray, 2006; National research council, 2005). The particular level of fibre intakes varies between countries due to analytical methods used to measure fibre content and accepted definition of what constitute a dietary fibre (Viebke et al., 2014).

Prebiotic is defined as non-digestible food ingredients which beneficially affect the host by selectivity stimulating the growth and/or activity of one or limited number of bacteria in the colon and thus improve the host health (Gibson & Roberfroid, 2013). These acts to promote beneficial effects by prebiotic including laxation, blood cholesterol attenuation and blood glucose attenuation with indication also that they can have potential effect in specific diseases, such as constipation, inflammatory and malignant colonic diseases (Phillips et al., 2008). Most agreed that the mediating agents in the physiological action in the host are the Short Chain Fatty Acid (SCFA) which is end products of prebiotics fermentation in colon. Reliance to the above definition of prebiotic it is clear that all the prebiotics are therefore dietary fibre but the reverse might not always be true.

2.8.2 Potential health benefits of Gum arabic

The act of Gum arabic as prebiotic and it is allegation as health beneficial substance has been reported on several occasions. It has been shown to be a

bifidogenic dietary fibre and fermented by intestinal bacteria to short-chain fatty acid, which associated with the health benefit (Cherbut, Michel, Raison et al., 2003). A study suggested that dose of 15grams of Gum arabic per day caused an increase in stool wet weight and water content in healthy adults (Cherbut et al., 2003) which beneficial to people suffering from constipation (Bliss, Stein, Schleifer et al., 1996). Another study claimed that the supplementation of 25 grams of Gum arabic per day result reduction in total serum cholesterol concentration (McLean Ross, Eastwood, Brydon et al., 1983; McLean Ross, Eastwood, Brydon et al., 1984). These observations was confirmed also by another study carried by (Shama, 1985). The suggested mechanism of gum arabic in lowering cholesterol levels is the through the surface adsorption of hydrocolloids in the small intestine or binding between the hydrocolloids and bile salts for example particularly for hydrocolloids such as Gum arabic that contain arabinogalactanprotein (AGP) (Viebke et al., 2014). Other studies indicated that suitable concentration of Gum arabic could be valuable in controlling or preventing diabetes (Shama, 1985). More studies revealed the beneficial effects of Gum arabic on the blood pressure, whereas, the relationship between blood pressure and the risk of cardiovascular mortality is well-established (Stamler, Stamler, & Neaton, 1993). Down to the same line further studies conducted on groups of normal individual and diabetic nephropathy where were both supplemented with 25 grams of Gum arabic for period of 8-12 weeks, the findings showed meaningful fall in the mean systolic blood pressure (SBP) which is seen in the both groups. Therefore, this effect in the blood pressure will translate into positive improvement in renal status because there is well-established correlation between progressions of renal disease and blood

pressure (David, Kazunari, & Aled, 2009). Additionally, the fermentation of Gum arabic in the large intestine to short chain fatty acid (SCFA) result in increasing of nitrogen excretion in the faeces which is thought to contribute to symptomatic improvement of patients with chronic renal failure (Bliss et al., 1996).

In dentistry, Gum arabic is also suggested to have an ability to enhance remineralization due to its high concentration of calcium ($Ca2^{+}$). Studies to evaluate the cariostatic activities of Gum arabic by using histopathological method concluded that the exposure of human molar to 10mg/m of Gum arabic resulted in significant remineralization of caries (Onishi, Umemura, Yanagawa et al., 2008). Moreover, the protective effect of gum arabic against enamel erosion was also studied by exposed the enamel samples to different concentration of polymer-modified citric acid. The study concluded that enamel samples treated with Gum arabic-enriched citric acid showed higher hardness and smoother surface (Seema & Arun, 2015) and that due to the act Gum arabic a as protective layer on the eroded enamel surface (Beyer, Reichert, Heurich et al., 2010).

2.8.3 Food applications

Gum arabic is unique substance and it has remarkable diversity of applications, due to it is desirable physicochemical and functional properties, such as emulsifier, stabilizer, thickener, formulation facilitate, surface finishing agent, firming agent, texturizer, adhesive, and recently as food dietary fibre (see Table 2-2). Gum arabic has been widely used for so many years in beverages and flavours industry, and that because Gum arabic has outstanding emulsifying, stabilising and acid stability properties. Beverage emulsion is a unique class of

emulsion compared to all other food dispersion system, virtually all beverages and flavours are macro-emulsions employ bio-polymeric emulsifier; Gum arabic is the most commonly used emulsifier. A high concentration of Gum arabic is required to stabilise a beverage emulsion as the oil droplet surface should be fully covered by the high molecular weight fraction rich in protein (AGP), to prevent flocculation and coalescence; for instance, the extensively used of Gum arabic to prepare and stabilise essential oil emulsion such as citrus flavour, cherry and cola (Matthias, 2010). Gum arabic also an ideal carrier in flavour encapsulation, liquid, solid or gaseous, because of its natural emulsifying and surface active properties, good retention of volatile flavour components; high solubility in water (up to 50%), acid stability, low viscosity and it is ability of forming a protective film to avoid penetration of oxidising agent, and allowing controlled release. It is useful technique to convert liquid food flavours to flow-able powder that can be used in dry food products (McNamee, Riordan, & Sullivan, 1998). Therefore, Gum arabic is used widely in encapsulation of aromatic composition, fruit juices, soups, dessert mixes, vitamins, enzymes, acids polyunsaturated fatty acids, trace elements, mineral oil and pesticides. Gum arabic is also widely used in confectionery industry to preform different properties; in candies it used to provide the appropriate texture which are easily deformed in the mouth but not adhere to the teeth, in lower-calorie candy Gum arabic is used to recompense the losses of texture, mouthful, and body, resulting from the replacement of sugar by artificial sweeteners (Brucker, Uhlarik, Lampe et al., 1974); in chewing gum it used as coating agent and as pigment stabilizer (Cherukuri, Friello, Parker et al., 1983), Gum arabic is also used in toffees and caramels as an emulsifier, to maintain a uniform distribution of the fat across the

product; in jelly products, Gum arabic is used to provide a fibrous, fruit like texture (Shigeo, Hiroya, Sumio et al., 1989). In the baking industry, Gum arabic is used for it is comparatively low water-absorption properties, in addition it has favourable adhesive properties for use in glaze and toppings and imparts smoothness when used as an emulsion stabilizer (Glicksman, 1983), furthermore gum arabic as a lubricant and binder is used widely in extruded snack cereals, moreover, it is also used to binds water to help retain humidity and control moulding and rolling properties in high sugar icings.

Furthermore, in wine Gum arabic is used to produce a clarify that it higher than can be obtained with other hydrocolloids (Williams & Phillip, 2000), in addition Gum arabic is used in wine to prevent sucrose crystallization, provides a controlled flavour release, and slows down melting in the mouth, making the wine gum long-lasting (Imeson, 1992). In beer Gum arabic is used as a foaming agent and to assist lacing (Tiss, Carriere, & Verger, 2001).

2.8.4 Enhancement of vegetable and fruit shelf-life

The dissolution of vegetable and fruit is main concern for the industry and consumers, to over-come this issue and delay further ripening a widespread method is conduct to avert the above issue by coating the fruit and vegetable with an edible coating that comply with the additive regulations. *Acacia* gum is one of the coating that used successfully to enhance the shelf-life of fruit and vegetable. A composite of 10% of Gum arabic and 1% chitosan used to make an edible coating, which commercially suitable for extending the storage life of bananas up to 33 days (Maqbool, Ali, Alderson et al., 2011). This shelf-life prolongation is supposed to be due to the reduced rate of respiration and ethylene evolution. Also, coating tomato with 10% *acacia* gum shows a significant delay

in changes of weight firmness, titratable acidity, soluble solids concentration, ascorbic acid and colour development (Idris, Ibrahim, & Mariod, 2013) .

In tropical fruit industry, the management of anthracnose is a major concern because of the resulting financial losses, combined of *acacia* gum with essential oils such as lemongrass or cinnamon showed antifungal effect and inhibition of the mycelia growth and spore germination (Seema & Arun, 2015).

2.8.5 Pharmaceutical applications

Gum arabic was extensively used in a variety of pharmaceutical products because of it is many functional properties such as binder, adhesive and glaze. Nevertheless, it now replaced by cellulose and modified starch in many applications, but it is still used as emulsifying and suspending agent in medicated cough drops and lozenges, also used as adhesive and binder and glaze for tablets (Tame-Said, 1997). In demulcent syrups, it is used for it is soothing and protective action, suspending agent and emulsifying agent (Ferdinand & Kruger, 1986; Millard & Balmert, 1961).

In cosmetics, Gum arabic functions as stabilizer in lotions and protective creams, where it increases viscosity, imparts spreading properties, and provides a protective coating and smooth feel. It is also used as an adhesive agent in blusher, and as a foam stabilizer in liquid soaps. Furthermore, gum is also used in the formulation of mascara, facial moisturiser, hair spray, eyeliner, body wash, lipsticks, and others (Whistler, 1993).

2.8.6 Printing, inks and paints

In printing industry gum arabic is used in the preparation of etching and plating solution in the lithography (FAO/WHO, 1995). Gum solutions are also used to

generate a protective film to prevent the printing plate from oxidation during storage. In inks industry, Gum arabic used as an emulsifier or suspending agent, because of it is protective colloid properties. However, the traditional used of Gum arabic has been in water colours as a binder because it is highly soluble in water, low in viscosity and when it dries, forms thin layer that binds pigments to the paper surface. In paints formation Gum arabic used as dispersant, keeping the pigments and active components uniformly distributed throughout the product (Fuyama & Tsuji, 1981; Gamble & Grady, 1938; Henson & Westveer, 1956).

2.8.7 Dual-functional gum arabic binder for silicon anodes in lithium ion batteries (LIB)

Silicon (Si) has attracted the attention of research and manufacturing as an anode material for lithium ion batteries because of high specific capacity (Etacheri, Marom, ELazari et al., 2011). The high cost and lack of effective mechanism to prevent the pulverization of Si electrodes during the lithiation / delithiation process and the lack of significant development in binders are obstacles that impedes industrialization and mass production of Si anodes (Meng, Xing-cheng, Gao et al., 2014; Shidi, Bin, Andrew et al., 2013). Gum arabic polymer known as a complex mixture of polysaccharides and glycoprotein. due to the unique structure Gum arabic, is applied as dual function binder, utilizing the concept of fiber-fiber reinforced in LIB electrode fabrication through the behaviour of the long glycoprotein chain in Gum arabic which acts like fibre. It is well-established that the fibre reinforces the rigidity, flexibility and toughness; and to enhance the tolerance to cracking from volume expansion in Si anode materials by forming Si and Gum arabic laminate due to exist of hydroxyl group in Gum

arabic which ensuring strong binding to Si (Lam & Teng, 2002; Samaan, Mirmiran, & Shahawy, 1998). The outcome of using gum arabic in Si anodes showed outstanding capacity and excellent long-term stability. Moreover, Gum arabic is natural low cost material and environmentally friendly polymer, benign a promising binder for lithium ion batteries (Min, Yana, Hui et al., 2015) .

Table 2-2. Summary of Gum arabic applications in various food products.

(Al-Assaf & Phillips, 2009).

Application	Necessary functionality and features needed for best performance
Emulsification in beverages	Coating of an oil droplet by the high molecular weight fraction rich in protein (AGP). Direct correlation between the proportion and molecular weight of AGP.
Encapsulation of essential oil: aromatic composition, fruit juices, vitamins, enzymes, acids polyunsaturated fatty acids, trace elements, mineral oil and pesticides	Forming a protective film to avoid penetration of oxidising agent, and allowing controlled release.
Confectionery	Preventing sugar crystallisation and emulsifying fat to ensure even distribution throughout the product. Long – term emulsion stability is not required particle for product with high sugar and low moisture content such as jujubes, pastilles, caramel and toffees. Thickening properties (viscosity) and film forming are requiring as a glaze and candy products. Binding agent for the paste base
Bakery for toppings and glazes.	Free flowing, adhesion properties, control the water absorption and to impart smoothness.
Texture and flavour modification in confectionery	Interact and bind water, to thicken as angel. Gel formation with enhanced water absorption. High proportion of AGP
Foam stabilisation – structure forming	‘’Lace curtain’’ effect on beer. Maximise content of high molecular weight component rich in protein which responsible for producing the foams.
Wine	Emulsifier and stabiliser for colour particularly in red wine by forming a protective film layer to prevent precipitation; reduce precipitation of acidity and tannin harshness; provide sensory impacts that include nose, palate and mouth feel modifications. Best performance achieved with high proportion of AGP to give long – term emulsion stability.
Dietary fibre	Dietary products, processed fruits, bakery items, frozen desserts, meat products and food for diabetics. Need ability to fermentation in colon to give short – chain fatty acids, with bulking ability.

Chapter 3

3. Materials and methods

3.1 Materials

3.1.1 Samples collections

168 authenticated samples of *A. senegal* var. *senegal* gum were collected from Sudan during 2008/2009 season. The samples were collected from six different plantations along the gum belt, all of which were preserved by the Sudanese National Forestry Corporation (SNFC). The records available at SNFC gave clear history of each plantation containing the age of the trees, locations and times of transplantation and seeding. The sampling covered the main two gum regions (i.e. eastern and western) in Sudan (see section 2.3.1) in six different states, where each plantation located in different state namely: Demokeye, Nabag, Twobon, Khor-Donia, Bozey and Trees belt. The exact location of each plantation on the Sudanese map together with the climate condition (taken from Sudanese Meteorological Authority (SMA) and SNFC) and details about the rain fall and soil type are given in Figure 3.1 and Table 3-1.

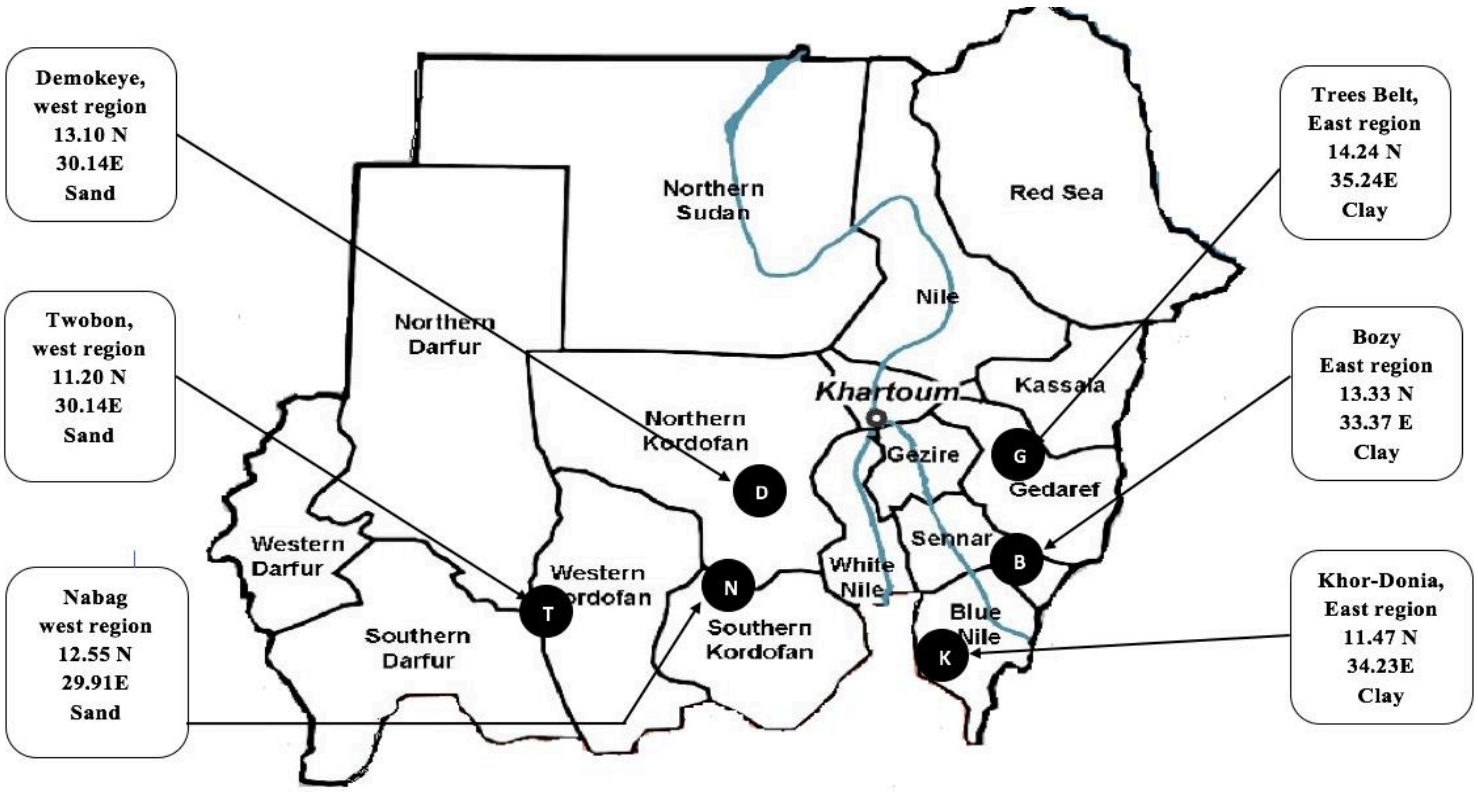


Figure - 3.1. The locations of plantations in Sudan- Where D is Demokeye, N is Nabag, T is Twobon, K is Khor-donia, B is Bozy and G is Trees-

Table 3-1. Samples location, soil type and climate conditions details (annual average rainfall, annual average temperature and annual average relative humidity) of the plantations used in this study. Source (SMA., 2013). where N.K is North Kordofan, S.K is South Kordofan, W.K is West Kordofan and B.N is Blue Nile.

Plantation	Location	Region	State	Soil	rainfall	temperature	humidity
Demokeye	13° 10′ N 30° 14′ E	West	N.K	sand	347 mm	27.3 C°	34.2%
Nabag	12° 55′ N 29° 91′ E	West	S.K	sand	490 mm	27.7 C°	39.8 %
Twobon	11° 20′ N 27° 49′ E	West	W.K	sand	500 mm	28.5 C°	37.5 %
Khor-Donia	11° 47′ N 34° 23′ E	East	B.N	clay	692 mm	28.3 C°	47%
Bozy	13° 33′ N 33° 37′ E	East	Sennar	clay	490 mm	28.6 C°	40 %
Trees belt	14° 24′ N 35° 24′ E	East	Gadaref	clay	610 mm	28.7 C°	43 %

The sampling procedure, conducted by the investigator over period of four months, followed carefully designed steps to ensure sample authenticity. This included the selection of plantation, labelling, tapping, collection and subsequent storage as shown in Figure 3.2 below.

Figure – 3.2. The sampling procedure (labelling, tapping and collecting) in different plantations conducted by the investigator with the help of professionals from the Sudanese National Forestey.

The plantations differ in the area size whereby some are widely extended, e.g. Twobon, Nabag, Khor-Donia and Demokeye. Additionally, some of these plantations are considered as pure plantation fully dominated by *A*. *senegal var*. *senegal* species, e.g. Demokeye, and Twobon while the remaining are considered as mixed plantation with different species of *Acacia* such as *A*. *seyal*, *A*. *polyacantha* and *A*. *mellifera*. However, the plantations used in this study were all dominated by *A*. *senegal* approximately 75%, e.g. Khor-Donia, Nabag, Trees Belt and Bozey.

In each area (plantation), with the help SNFC staff the sampling was conducted to collect two types of samples. Firstly, according to the age of the tree where four different set of ages: namely 5-10 years, 11-15 years, 16-20 years and over 20 years old.

84 specific samples were then collected from specific tree of specific age as given above. Additionally, another 84 representative samples were also collected by mixing the gum

collected from 7-8 trees of the same age set. In both cases the tree was clearly labelled with red paint (see Figure 3.3).

Figure - 3.3. Sampling process, labelling of selected *A*. *senegal* trees.

The representative sample was collected from seven trees or more in each plantation for each age set throughout the interval collections. The selection of the trees was based on yield of the tree and general health. The process of tree age identification was based on the Sudanese National Forestry Corporation (SNFC) records and documents available in the location of the respective plantation. Unlike other trees *Acacia* trees do not possess growth rings to help estimate the age. Therefore, the range of five years was used in each set of ages depend on the way of (SNFC) and time of transplantation and seeding the *Acacia* trees. It should be noted here that re-seedling of the same area over five years until it fully covered by the trees is the usual practice and hence certain areas in the plantation contain a range of five years of trees. For the above reason the age range was given as 5-10, 10-15 and so on. For ease of presentation and labelling the tree age used in this thesis will use the lowest number for each set, i.e. 5, 10, 15 and 20 years.

Moreover, the samples also collected from four different picking intervals (see Table 3.2). However, it was not possible to obtain samples from the fourth pick in Bozy plantation.

It should be also noted that the plantations were all guarded and managed by two guards and one forest observer during the whole period of sampling which continued for 4 months.

Table 3-2. Samples details and total number (T) of samples collected from each planation.

Code	Plantations	Region	State	Soil	Picks	Age sets	T.samples
D	El-Demokeya	West	N.Kordofan	Sand	4	4	16
N	Nabag	West	S.Kordofan	Sand	4	4	16
T	Twobon	West	S.Kordofan	Sand	4	4	16
Kh	Khor-Donia	East	Blue Nile	Clay	4	4	16
B	Bozey	East	Senar	Clay	3	4	12
G	Trees belts	East	Gadareef	Clay	4	2	8
Total							**84**

3.1.2 Samples labelling

In each of above mentioned plantations samples were collected from one specific tree (of certain age) as well as representative samples from the same age and location during various picks throughout the season. The following method was used: the trees from each age set were labelled by using red paint and coated label, each label containing serial number, set of ages, time of tapping and samples code. The labels were tied strongly onto highest possible branch of the tree to avoid being removed by wind or animals. The codes in the label for each sample contained the following information: name of the plantation (the first letter from each plantation.*)*, type of samples (i.e. from specific tree or representative trees), age of the trees and number of collections (pick).

For examples the codes DT5P1, DR5P2, used in the samples list in chapter 4, refer to the following:

D: refers to the plantation name (Demokeye) for both codes.

T: indicates specific tree and R means representative (sample from a group of trees).

5: is the age set of the trees age 5-10 years.

P1: indicates the first pick after tapping and P2 is second pick and so on.

The specific tree from each age set was been chosen by the agricultural expert from (SNFC) in each plantation. The representative trees were randomly selected from the same age set from the different trees representative the sample of whole area.

3.1.3 Samples preparation

The samples were collected in pre-labelled small cotton bags and were then poured into a plastic dish and dried in an open area under shelter to avoid the direct sum light for a period of one week as shown in the example in Figure 3.4. Impurities such as bark and sand were removed following drying.

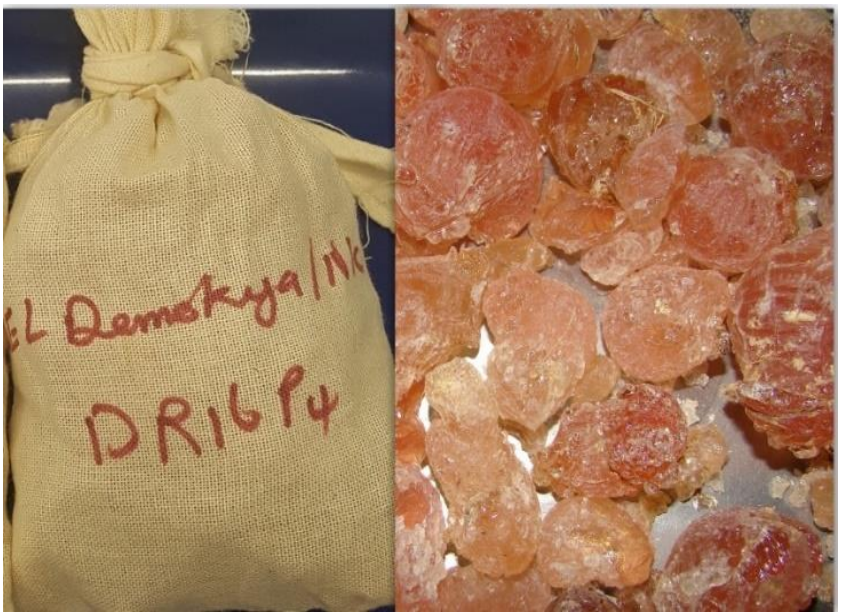


Figure - 3.4. Example of the method used for collecting and drying the gum samples used in this study.

Samples were then further re-cleaned and kept in the form of mixed lumps and nodules with small broken gum to mimic the actual established method of sample preparation used by the industry in the preparation of the widely known commercial great of cleaned gum (KC) (see section 2.6.4). Samples were packed again in the same small cotton bags and allowed to further dry for 10 days in the open-air environment with occasional exposure to direct sun light. Further cleaning was also carried out to remove any residual barks and impurities. Samples were later kibbled to less than 1mm size by using pestle and mortar, and grinder machine, just before they used to conduct the tests.

3.1.4 Samples storage

Each sample was then divided into three portions in labelled cotton bags. The first portion was immediately brought to the UK (fresh samples) to undertake the tests on the freshly prepared samples and was stored in the same bags in a metal cabinet in the laboratory and called UK stored samples for three years (UK-S-3) and for five years (UK-S-5). The two remaining portions were stored at different locations in Sudan. The second portion was stored at Khartoum for five years (Kh-S-5), at a gum factory, where the weather tends to be hot and dry. The third portion was stored at Port Sudan for five years (PS-S-5) where the weather is very hot and humid. The three portions of samples were stored for five years.

3.2 Methods

3.2.1 % loss on drying

The percentage loss on drying (otherwise widely reported as moisture content) was determined according to the method of (JCEFA-FAO, 1999) as follows. An empty aluminium dishes were dried in convection oven (SANYO MOV – 212F) at 105 ℃ for 30 minutes, cooled in desiccator and weighed (W_1). About 1 gm of well mixed gum powder were placed in the aluminium dishes and weighed accurately (W2) and heated for 5 hours at 105 ℃, cooled in the desiccator and weighed again (W3). The loss on drying was calculated according to equation 3-1 below

$$\% \text{ Loss on drying} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \qquad [3\text{-}1]$$

Where, W_1 is weight of the empty dish, W_2 is weight of sample + dish before drying and, W_3 is weight of sample + dish after drying. The measurement was carried out in duplicate and the average value is reported. In this thesis, the term moisture content will be used to refer to this measurement.

3.2.2 Specific optical rotation

3.2.2.1 Background

Interaction of electromagnetic radiation with matter produces several observable effects such as absorption, scattering, refraction, and rotation. The latter phenomenon is commonly known as optical rotation which is produced by chiral molecules, when a carbon atom is bonded to four different groups and produce mirror image (isomers) of a particular molecule. Optical rotation is one of the physicochemical properties associated with the structure of organic molecules, biopolymers and proteins. In principle, the rotation

produced by a molecule is equal in magnitude but opposite in sign to that produced by mirror image. An assembly of molecules with freedom of rotation, such as that which occurs in solution, will produce an observable net effect only if the individual molecules are optically asymmetric. They cannot be converted by rotation into their isomers. The direction and magnitude of rotation are sensitive to the detailed structure in the vicinity of the asymmetric centre of the molecule. The rotation may be either clockwise, to the right (dextrorotary - d-rotary), or left (levorotary -l-rotary) depending on which stereoisomer is present (or dominant).

3.2.2.2 Method

The specific optical rotation was determined according to the method of (JCEFA-FAO, 1999). A 0.10 g/mL aqueous solution of the respective gum was prepared as follows. Around 10.0 g of test material was accurately weighed into a glass bottle. The sample was dissolved in around 40 g of distilled water, accurately weighed, mixing on a roller mixer until the sample fully dissolved (approximately 5 hours). The precise concentration (% (w/w)) was calculated based on the loss on drying. About 25 g of concentrated sample solution (~20 % (w/w)), accurately weighed, was transferred into a 50-ml volumetric flask, and diluted to volume with distilled water. The solution was filtered through 0.8 μm cellulose acetate membranes (Naglene, 25mm).

Optical rotation was measured using a 200-mm tube filled with the test solution at room temperature on a Polari-meter AA-5 (Optical Activity Ltd). Specific optical rotation was calculated by the following equation 3-2.

$$[\alpha]_{\lambda}^{T} = \frac{\alpha_{\lambda}^{T}(^{\circ})}{l(dm) * C(g/ml)} \qquad [3\text{-}2]$$

Where, α is the observed rotation of the solution in circular degrees, *C* is the grams of substance per 1 ml of solution, and L is the length of the solution in decimetres.

3.2.3 Protein content

3.2.3.1 Background

The spectrophotometric methods for protein measurement have great advantages over the other procedures in simplicity, rapidity and sensitivity. One of the most frequently employed methods for protein determination is the Lowry method. However, the method has number of disadvantages such as the standard curve is nonlinear and the technique uses instable regent which need to be prepared daily. The Lowry method is derived from the biuret reaction which based on the reaction of a mixture of copper sulphate and sodium-potassium tartrate with protein. Then folin-Ciocalteu is added. A blue-purplish colour forms that is measured at 595 nm (Lowry, Rosbrough, Farr et al., 1951). Lowry method is reviewed by Lane and Peterson (Lane, 1957; Peterson, 1979, 1983).

The Lowry method involves two reactions:

1- Formation of protein-Cu^{2+} complex. Six peptide bonds surround a central Cu^{2+} atom in alkaline solution. The high pH solvent (~ pH 13) induces protein denaturation. The loss of native structure precedes binding with Cu^{2+} to form type of protein $-Cu^{2+}$ complex as illustrated in Figure 3.5.

$$Cu^{2+} + \text{protein} \rightarrow Cu^{2+} \text{ Protein complex.}$$

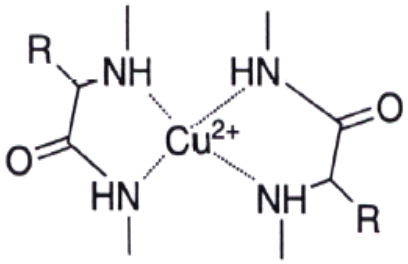


Figure - 3.5. $Cu2^{+}$-protein complex.

$$Cu^{2+} + (\text{polar amino acids})_{red} \rightarrow Cu^{+} + (\text{amino acids})_{ox.}$$

Is redox with the folin-Ciocalteu regent, where Cu2+ and radical groups of the amino acid reacted with folin – ciocalteu regent via two pathways; first react directly with amino acid side chains (Histidine, cysteine, asparagine, tyrosine and tryptophan). Second Cu^{2+} mediates the dehydrogenation of the polypeptide via metal ion catalyzed oxidation. The electrons are transferred into the solution yielding a Molybdenum blue colour change proportional to the protein concentration (Chuo et al, 1960, Legler et al, 1985).

3.2.3.2 Method

Stock solutions for reagent A, B, C, D and E were prepared as described below.

Reagent A (2% sodium carbonate in 0.1M sodium hydroxide); Reagent B (1% copper sulphate containing 2% sodium potassium tartrate); Reagent C (alkaline copper tartrate contains mix of 50 ml of reagent A and 1ml of reagent B. Prepared daily).

98 ml of reagent A and 2ml of reagent B mixed gently; reagent D (Folin – ciocalteu reagent). diluted 1:1 with distilled water. Reagent E (Phosphate buffer PH 7.2) prepared by adding 8g of sodium chloride, 0.2g potassium chloride, 0.24g potassium phosphate and 1.44g sodium phosphate to 1000 ml of distilled water.

Standard: Bovine serum albumin (BSA, obtained from Sigma-Aldrich), or other protein similar to sample protein in order to construct the calibration curve. The standard dissolved (overnight) at a concentration of 2 mg/ml (2000 µ l/ml) in phosphate buffer (pH 7.2) and diluted to different concentrations (1500, 1000, 500 and 250 µ l/ml); 10 ml of samples of 2.5% solution were prepared as a solvent and mixed overnight in roller mixture. Subsequently, 0.3 ml of test samples taken in universal tube and then 0.3 ml of reagent (C) added to each tube and mixed gently, the mixtures incubated for 30 minutes at room temperature. 0.3 ml of folins reagent (D) added to each tube and mixed gently and then incubated for 30 minute at room temperature. The reading of the absorbance of standard

and samples were measured using UV-Vis spectrophotometer (Perkin Elmer Lambda 25 UV/Vis systems) at 595nm. The calibration curve was then constructed and the equivalent protein content in the respective sample was then determined. Typical calibration curve is shown in Figure 3.6 below.

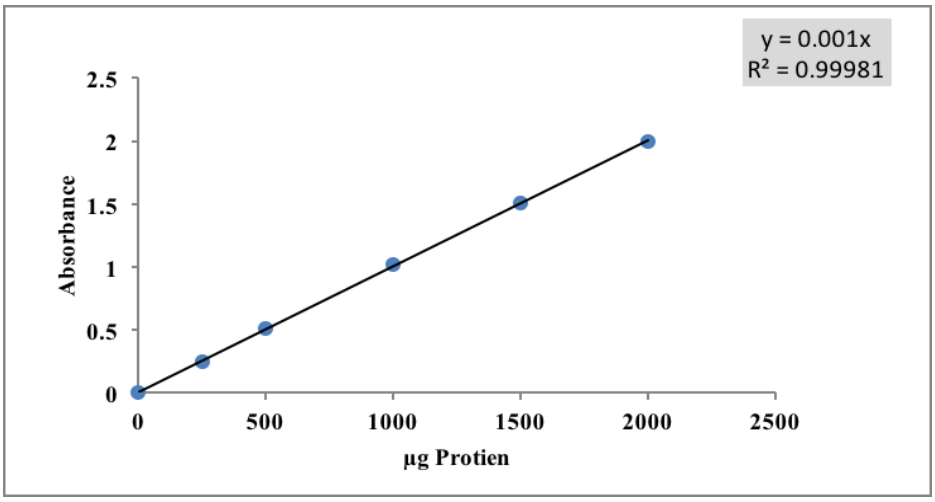


Figure - 3.6. Typical Lowry calibration standard curve.

The following equation 3-3 was then used to calculate the concentration of the protein.

$$\text{Protein \%} = \frac{Abs}{C \times Sl} \times 100 \qquad [3\text{-}3]$$

Where:

Abs is the absorbance reading from UV-Vis spectrophotometer at 595 nm

C is the concentration of the sample µg/ml

SL is the slope from the calibration curve

3.2.4 Intrinsic viscosity

3.2.4.1 Background

The intrinsic viscosity or more accurately defined as the limiting viscosity number (η), is one of simplest and cheapest method employed by many polymer scientists. It is a relative method, based on the principles that bigger polymers molecule make solution more viscous than small ones do. The intrinsic viscosity is based on the time it takes certain volume of polymer solution flow through a thin capillary compared to the time for solvent to flow (Al-assaf, 2016) . It is used to measure the viscosity of diluted polymer solution and represents essentially the volume occupied by polymer per unit mass (cm^3/g or dl/g). The intrinsic viscosity is determined by measuring the diluted polymer solution efflux time between fixed marks in glass capillary viscometer. Figure 3.7 shows the Cannon Ubbelohde Cabillary viscometer used in this study.

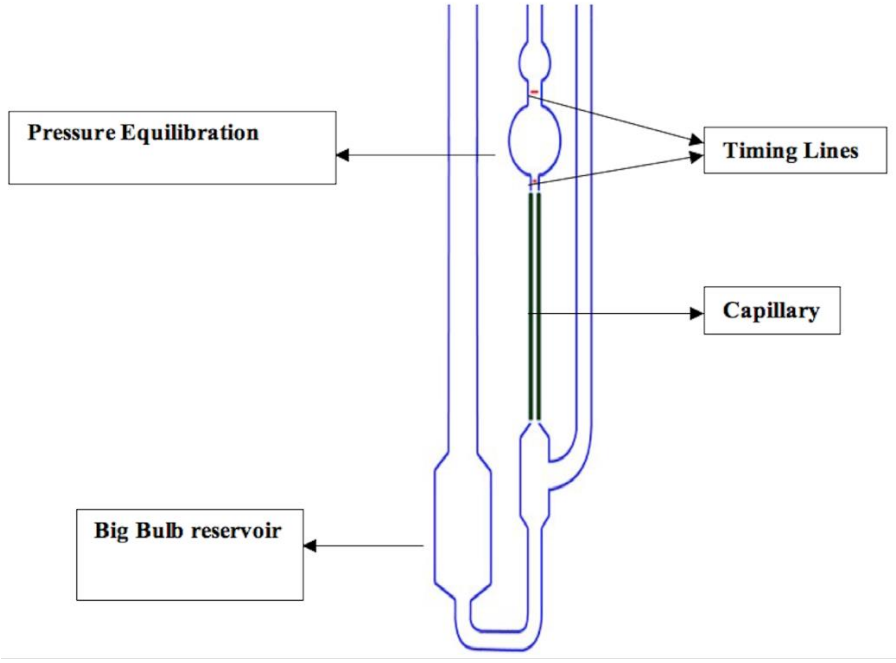


Figure - 3.7. Cannon Ubbelohde Capillary Viscometer.

The quantity from intrinsic viscosity provides a wealth of information relating to the size of polymer molecule in solution, including the effect of polymer structure, molecule shape and degree of polymerization and polymer solvent interaction. In a good solvent, the energy of the interaction between a polymer segment and solvent molecule adjacent to it exceeds the mean of the energies of interaction between the polymer – polymer and solvent – solvent pairs, the polymer will tend to expand further so as to reduce the number of contacts between pairs of polymer element, therefore, the polymer molecule will be in a very extended form, and the value of intrinsic viscosity will be high. On the other hand, in the poor solvent the energy of interaction between a polymer segment and solvent molecule is very small, in which polymer – polymer contacts occur more frequently, and the polymer will tend to occupy a tightly coil form, resulting in lowering of intrinsic viscosity (Amalvy, 1997).

For the dilute polymer solution, the density term is assumed to be that of the solvent. At least four dilutions can be made in situ using the solvent. The relative viscosity for the lowest and highest concentrations should be in the region of 1.2-2.0 to ensure that all measurements are in diluted region. By measuring the efflux time of solvent and the efflux time of the polymer solution, thus giving relative viscosity of a polymer solution (η_r) shown in equation 3-4.

$$\eta_r = \frac{t_{solution}}{t_{solvent}} \qquad [3\text{-}4]$$

Where $t_{solution}$ and $t_{solvent}$ are the flow times of the polymer solution and solvent, respectively. The specific viscosity, η_{sp} which is measure of the relative increment in the viscosity caused by the dissolved polymer can be obtained from Equation 3-5:

$$\eta_{sp} = \frac{t_{solution} - t_{solvent}}{t_{solvent}} = \eta_r - 1 \qquad [3\text{-}5]$$

The reduced viscosity (η_{red}) and inherent viscosity (η_{inh}) may be defined as follows:

$$\eta_{red} = \frac{\eta_{sp}}{C} \qquad [3\text{-}6]$$

$$\eta_{inh} = \frac{\ln \eta_r}{C} \qquad [3\text{-}7]$$

where c is the polymer concentration (mg/dL). If relative viscosity measurements are made under constant high ionic strength and by extrapolating the plot of the reduced or the inherent viscosity against concentration to infinite dilution· The intrinsic viscosity can be obtained in the same way as for uncharged polymers as given in Equation. (3-8).

$$[\eta] = \lim_{c \to 0} \eta_{red} = \lim_{c \to 0} \eta_{inh} \qquad [3\text{-}8]$$

Experimentally, a plot of reduced viscosity as a function of concentration will give a straight line with an intercept equal to the intrinsic viscosity as given by the Huggins equation, Equation 3-9 (Huggins, 1942). Alternatively, the intrinsic viscosity could be obtained by plotting the inherent viscosity as a function of the concentration as shown by Kraemer's equation, Equation 3-10.

$$\frac{\eta_{sp}}{C} = K'[\eta]^2 C + [\eta] \qquad [3\text{-}9]$$

$$\frac{\ln \eta_{inh}}{C} = K''[\eta]^2 C + [\eta] \qquad [3\text{-}10]$$

($K'$is Huggins constant and K'' is Kraemer constant)

A representative Huggins and Kraemer plot to determine intrinsic viscosity is shown in Figure 3.8. Generally, Huggins and Kraemer plots intersect at zero concentration, as shown

in Figure 3.5, and the average value of the intercepts is used as the intrinsic viscosity. However, in the presence of aggregation, the Huggins and Kraemer plots will not intersect at zero concentration (Sperling, 2006).

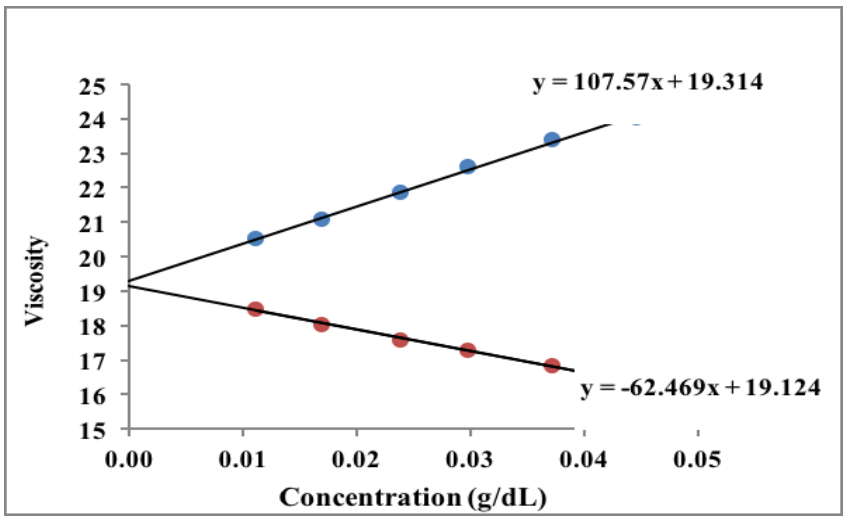


Figure - 3.8. Representative Plot of reduced and inherent viscosities as a function of concentration for the determination of the intrinsic viscosity of *A. senegal* gum 0.1M NaCl at 25°C.

In such cases, the value of the intercept of the Huggins plot at zero concentration is often used as the intrinsic viscosity. Huggins and Kraemer constants can be calculated with following equations:

$$K' = \boldsymbol{d}_{Huggins} / [\eta]^2 \qquad [3.11]$$

$$K'' = \boldsymbol{d}_{Kraemer} / [\eta]^2 \qquad [3.12]$$

where d is the slope of the curve. The value for the Huggins constant is approximately 0.4 for many completely water soluble polysaccharides, and any higher values (>1) are usually taken as an indication of aggregation [3-9,3-10] (Al-assaf, 2016) . The limiting viscosity number or intrinsic viscosity [η] is an index of the size of isolated polymer coils, and it has units of reciprocal concentration. The viscosity-average molecular weight (M_v) can be

evaluated from viscosity data if a correlation can be established between [η] and the weight-average molecular weight. This approach requires knowing or being able to determine the appropriate K and a value in the Mark–Houwink equation:

$$[\eta] = KM^{a} \qquad [3\text{-}13]$$

Where, M is the viscosity average molecular weight, K and ‘’α’’ are the Mark-Houwink constant for a particular polymer in given solvent at given temperature. The K and ‘α’ parameters are usually determined by a plot of log [η] versus log of weight-average molecular weight (M_w). Anderson and Rahman (1967) determined the value of Mark-Houwink constants *K* and *α* for *A*. *senegal* gum and reported the value of K= 0.013 and α = 0.54. Measurement carried out at 25 C with1.0 M Nacl as solvent. Each of the two parameters, *k* and *a* reflects a combination of contribution. K is related essentially to the intrinsic viscosity or local chain flexibility, including the orientation of the bonds to form constituent monosaccharide. When the exponent *α* can reflected the chain geometry, branched, sphere, rod or coil and solvent quality (Young & Lovell, 1991)· The *α* value is an effective parameter of polymer conformation and polymer–solvent interactions. If a is 0.6−0.8, the polymer is in random coil configuration in solution, and if the α value is greater than 0.8, the polymer behaves as a rigid rod. A value of zero indicates a spherical conformation, see Table 3-3. (Al-assaf, 2016) .

Table 3-3. Mark-Houwink coefficients for three extreme chain conformations.

Coefficients	compact sphere	random coil	rigid rod
α	0	0.6~0.8	< 0.8

3.2.4.2 Method

The relative viscosity was determined using capillary viscometer Ubbelohde serial No (75 N 104 & 94). The test material was dissolved in 1.0M NaCl solution to give solution with concentration of 4%. The solutions were filtered through (Nitrite cellulous membrane 0.8 μm) filter into a clean universal. The viscometer was placed in the holder and introduced in to a constant temperature water bath set at 25C°. The viscometer was cleaned by washing with distilled water and followed by the addition of acetone which was then evaporated by blowing compressed air or nitrogen through the viscometer. The flow time of solvent was measured ($t_{\circ}$). Exactly 2ml of the respective sample were introduced in to the reservoir of the viscometer using 2ml pipette and the flow time of the sample was measured (t). The dilute polymer solution introduced in to the viscometer through the large bore tube in to the lower reservoir, the solution was allowed to equilibrate for (2-5min). The solution was then pulled by suction to the upper marked line and the time recorded till it reaches the lower mark line. Duplicate measurements were performed and the average was taken. Representative intrinsic viscosity graph is shown in Figure 3.8.

3.2.5 Gel Permeation Chromatography – Multi-Angle Laser Light Scattering (GPC-MALLS) analysis

3.2.5.1 Background

Gel filtration chromatography (GFC) also known as gel permeation chromatography (GPC) or size exclusion chromatography (SEC) is a type of liquid chromatography first introduced by Moore and Hendrickson in1960s. GPC is primarily used to obtain molecular weight distribution information for polymeric materials. It provides information about the molecular weight, i.e. number, weight and Z averages (M_n, M_w, M_z) as well as the molecular weight distribution (and polydispersity). In this technique, the polymer molecule is separated according to their molecule size or (hydrodynamic volumes) in solution. As the

solvent elutes through a column packed with a porous material (stationary phase). When a sample is injected into a GPC column, polymer molecules larger than the pores of the packing material cannot enter the pores and are eluted at the interstitial volume V_i (also known as the void volume of the column). It should be noted that no fraction of the sample can be eluted before the interstitial volume has passed through the column. Small molecules, however, are able to diffuse in to the pores where the larger molecules are excluded, will therefore elute at the sum of both the interstitial and pore volume, i.e. $V_i + V_p$. Molecules that have sizes between the above two extreme values will have access to only a part of the pore volume and will therefore be eluted at the elution volume V_e.

$$V_e = V_i + K_{SEC}\ V_p \qquad [3\text{-}14]$$

Where V_e is the elution volume, V_i is the interstitial volume and V_p is the pore volume. K_{SEC} is the equilibrium constant of a sample in size exclusion chromatography ($0 < KSEC < 1$).

GPC is relative technique and requires standards of certain molecular weight (determined by an absolute technique such as laser light scattering) to be injected so that the elution volume can be determined and used to compare with the test materials.

Laser light scattering (LLS) is one of the few absolute methods available for the determination of molecular weight and size over broad range. The phenomena of scattering of the light by the molecule can be explained as the excitation of the atom or molecule. When a beam of electromagnetic waves strikes atoms or molecule in the center, the wave provides the atoms or the molecules with more energy, due to the electrons being displaced and oscillate about their equilibrium position with the same frequency as the incident exciting beam. This forces the electron to move to one direction and the nuclei to the

opposite direction, which result in an induced transient dipole in the atoms or molecules. As a consequence, the atoms or molecules will act as secondary scattering center by re-emitting the light in all direction. Larger molecules give larger intensity of scattered light. The larger particles or polymer molecule are made up of monomer units which can act as secondary poly-scattering centers, contribute on the intensity of light scattered at a certain angle. Light scattering can be affected by intermolecular interaction (interaction between two neighboring chains) producing a non-ideality. This non-ideality, the second virial coefficient, can be measured with light scattering using a series of different concentrations. In this study the measurements were carried out on dilute solutions to minimize these interactions since in this regime, the polymer molecules are separated by large distances (Al-assaf, 2016) .

Light of a uniform wavelength is emitted from a source, and passes through a glass cell containing the solution of interest, shown in Figure 3.9. Multi detectors are positioned at varying angles to simultaneously measure the intensity (I(θ)) of scattered light.

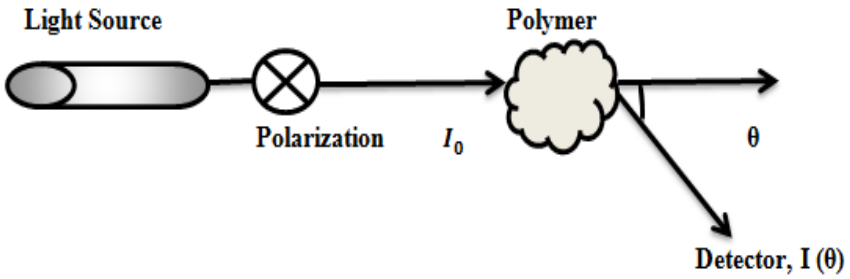


Figure - 3.9. Path of light scattered through a polymer solution.

The incident light (I_0) and scattered light (I (θ)) in light scattering experiments are related by the Rayleigh ratio (Equation 3-15).

$$R(\theta) = \frac{I(\theta)}{I_0} \quad [3\text{-}15]$$

The light scattering intensity of a polymer solution is proportional to the product of its molecular weight (M), concentration (C) and the specific refractive index value (dn/dc), as given in Equation (3.16)

$$I_{scattered} \propto M C (dn/dc)^2 \quad [3\text{-}16]$$

The intensity of the scattered light is related to the molecular weight of the molecules can be calculated using by Equation 3-17.

$$\frac{K^* c}{R(\theta)} = \frac{1}{Mw \times P(\theta)} + 2A_2 c + \cdots \quad [3\text{-}17]$$

Where:

c is the mass concentration of the solute molecules in the solvent (g/mL).

Mw is the weight average molecular weight (g/mol).

A_2 is the second virial coefficient (mol mL/g^2). Is a measure of macromolecular self-interaction, and is useful parameter for optimizing crystallization and formulation conditions of protein.

K^* is an optical constant as shown in Equation 3-18 below.

$R(\theta)$ is the excess Rayleigh ratio (cm^{-1}) of the solution.

K^* accounts for scattering effects due to laser wavelength, solvent and flow cell refraction and sample dn/dc.

$$K^* = \frac{4\pi^2 {n_0}^2 (dn/dc)^2}{{\lambda_0}^4 N_A} \quad [3\text{-}18]$$

Where:

n_0 is the refractive index of the solvent at the incident radiation (vacuum) wave-length.

λ_0 is the incident radiation (vacuum) wavelength, expressed in nano-metres.

N_A .is Avogadro's number, equal to 6.022× 10^{23}mol^{-1} .

dn/dc is the differential refractive index increment of the solvent-solution with respect to a change in solute concentration, expressed in mL/g (this factor must be measured independently using a DRI detector).

Equation (3-17) is only valid at all angles for small particles or molecules of dimensions of less than λ'/20, where λ' = λ0/n_0, as small particles scatter the light isotropically. This is known as the Rayleigh–Gans–Debye limit. Here, λ0 is the wavelength in vacuo of the incident beam and n_0 is the refractive index of the solvent (JACKSON & BARTH, 2004). The light scattering intensity exhibits an angular dependence for particles above 10 nm in size (i.e. molecules with a major dimension larger than λº/20 of the light's wavelength). This is usually the case in polymer solutions. Consequently, such variation depends on the size of the molecule, the wavelength of the light λ, and the observation angle θ. The Rayleigh-Gans-Debye approximation combines the light scattering principles of Equation. (3-17) and its angular dependence according to Equation. (3-19) (JACKSON & BARTH, 2004).

$$I_{scattered}\ (\theta) \propto R(\theta) = K^{*}Mc\ P(\theta)[1- 2A_2\ Mc\ P(\theta)] \qquad [3\text{-}19]$$

Where

M is the molecular weight;

C is the polymer concentration;

and P(θ) is the form factor, corresponding to the angular variation of the scattered intensity as a function of the mean square radius, *Rg*, of the particle. The larger the *Rg*, the larger the angular variation will be. A_2 enters the light scattering equation as a correction factor for concentration effects. The *dn*/*dc* is the specific refractive index increment, which is the change in the refractive index of a polymer solution relative to the change of polymer

concentration (see Equation. 3-18). The *dn*/*dc* value depends on the type of polymer; the type of solvent in which it is measured, and the wavelength, and it is required to accurately determine the molecular weight by light scattering.

The *dn*/*dc* can be obtained directly with a differential refractive index detector using a series of polymer concentrations and measuring the change in refractive index, Δn, at the wavelength of the incident beam. A plot of Δn as a function of concentration is then constructed and by extrapolation to zero concentration, the dn/dc value is obtained.

$P(\theta)$ is the particle scattering function which describes the angular variation of the scattering intensity by providing the ratio of the scattering intensity to the intensity in the absence of the interference measured at the same angle θ. $P(\theta)$ can be related to the specific shape of structural dimensions such as a coil, sphere or rod. At low scattering angles P_θ is given by Equation 3.20.

$$\frac{1}{P(\theta)} = 1+\left\{\frac{4\pi}{\lambda}\right\}^2 sin^2\{\frac{\theta}{2}\}\frac{\langle Rg\rangle^2}{3} \qquad [3\text{-}20]$$

Where

λ = the wavelength of incident light

$\langle RG\rangle^2$ = the mean square radius of gyration.

The various parameters such as Mw, Rg and A2, which obtained from the light scattering measurement, could be calculated by a variety of mathematical formulas that can be used, with the Zimm formula being the most commonly used, but Debye and Berry methods are also possible. These methods differ in the quantity used on the ordinate (Y axis) but the abscissa (X axis) is always the same, methods shown in Table. 3-4 below.

Table. 3-4 Light scattering measurement different analysis methods (Al-assaf, 2016).

Methods	Extrapolations	Intercept	Slop	Favourable
Berry	SQRT 0 $\hat{K}$ c /R (θ) *vs*. $\sin^2(\theta/2)$	Mw	rg^2	Useful for larger molecules
Zimm	$\hat{K}$ c /R (θ) *vs*. $\sin^2(\theta/2)$	Mw	A2	It works well for mid-sized molecules (rms radius ~20-50 nm).
Debye	R (θ)/ $\hat{K}$ c *vs*. $\sin^2(\theta/2)$	Mw	rg^2	It gives good results over a wider range of molecular weight as compared to the Zimm formalism.

Gel permeation chromatography (GPC) coupled to MALLS and refractive index detector (RI) is currently the best available technique for the absolute determination of polymer molecular weights and their distribution. In addition, the proteinaceous material can be detected by using ultraviolet detector (UV). The essential components of the GPC-MALLS instrumentation are illustrated in Figure 3.10. It consists of a solvent reservoir, a solvent delivery system (pump), different filters, sample injection system, gel packed column, elution detection system (composed of three detectors, Refractive index (RI), Multi angle laser light scattering (MALLS), and Ultra violet detector (UV)) and computerize data processing system. The heart of all the systems is gel packed column (GPC), where polymer fractionation occurs. The three detectors are monitoring the qualitative and quantitative properties of the eluting polymer molecules from the GPC column, the light scattering

detector utilizes the principle that the intensity of the light scattered elastically by a molecule (Raleigh scattering) is directly proportional to the molecule weight (mass detector), the refractive index detector connected directly after the light scattering detector to measure the concentration of each fraction as elutes from the GPC column, as a terms of the difference in refractive index between the solvent and solute, concentration detector and Ultra violet detector (UV) at 214 nm which specifically show the amount of the protein in the different fractions.

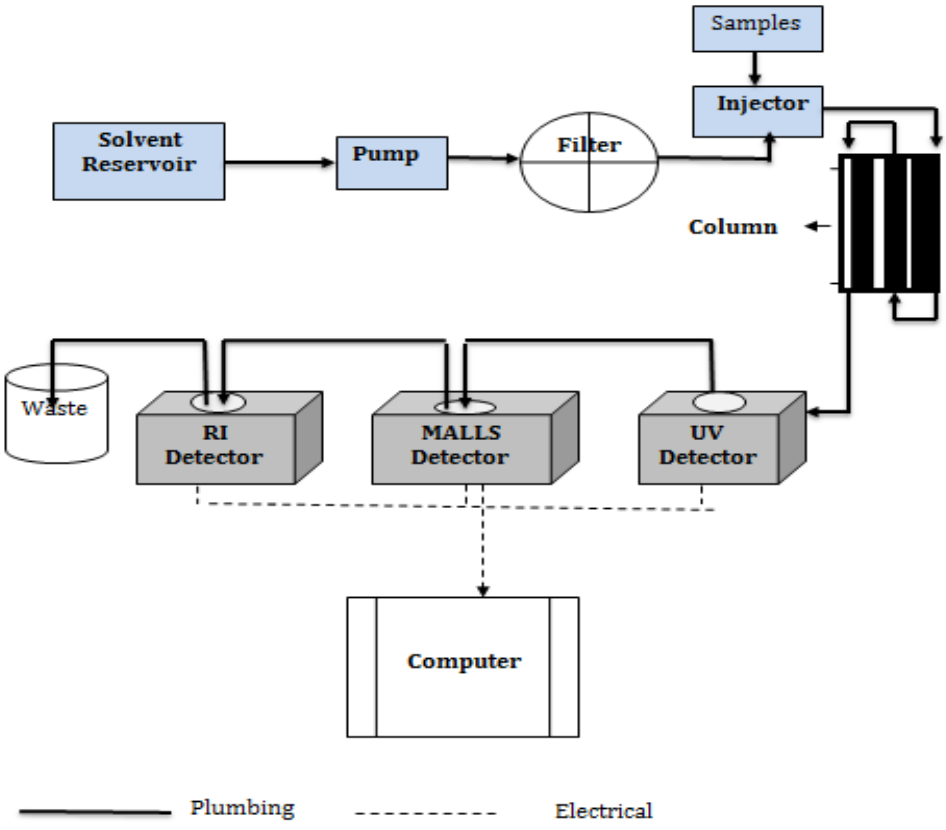


Figure - 3.10. Atypical GPC-MALLS flow diagram. Solvent enters the degasser and is pumped through in-line filter before the sample is injected and pass through columns and detectors.

3.2.5.2 Method

GPC-MALLS measurements were performed at room temperature to determine the molecular weight of the test material. 20 mg of test material was accurately weighted into plastic vial. Solid content of the sample was calculated based on the loss on drying. The sample was dissolved in 10 ml of the respective solvent (0.2 M NaCl) (the density of gum arabic was assumed as 1.0 g/ml). The solution was left on the roller mixer until the sample has fully dissolved (approximately 5 hours) and stored at 4 C° overnight and used the following day. Prior to measurement, the test solution was taken out from the fridge and left to tumble mix for at least 30 min at room temperature then filtered using a 0.45µm nylon filter (Whatman, 13 or 17mm). 0.2M NaCl solvent prepared and filtered through 0.22 µm (Millipore) as eluent at flow rate of 0.4 ml/min (Knauer, HPLC Pump K-501). An injection volume of 100 μl (Rheodyne 7725i) was used. Samples injected volume 100µl in to Superose 6HR 10/300 (Cross linked a garose) column supplier by (Amersham Biosciences).

Astra for windows software (version 4.90.07, Wyatt Technology corporation, USA) was used in the instrument control and data acquisition by an Agilent 1100 series G1314A UV detector (214nm, Agilent Technologies), a DAWN EOS multi-angle laser light scattering detector (λ = 690 nm, Wyatt Technology corporation) and an Optilab® rEX refractometer (Wyatt Technology corporation, USA). The differential refractive index increments (dn/dc value of 0.141 ml/g was used (R. C. Randall, G. O. Phillips, & P. A. Williams, 1989).

There is a total of 18 detectors in the DAWN EOS instrument. However, for aqueous solution the signal from lower angle detectors, i.e. detectors #3-4 and #18, is very noisy and for some samples these reading was deleted during data processing. The subset of signals from light scattering detectors #5 - #17 was used in the analysis. MALLS detector was

calibrated using distilled water, ethanol, air, toluene (SPECTTRANAL 99.9 %, Riedel-de Haën, Germany). The Pullulan standard from Shodex (molecular weight 47,000) was used to normalize the detectors and for the determination of the delay volume; the delay volumes of the equipment were 0.0296 ml between UV detector and DAWN EOS, and 0.1353 ml between DAWN EOS and refractometer. Refractive index detector was calibrated using NaCl solvent (dn/dc = 0.172) at different concentrations (5 x 10^{-5} g/mL – 1 x 10^{-3} g/mL).

3.2.5.3 Molecular weight processing

Gum arabic is a highly heterogeneous complex polysaccharide which consists of the three main components (see section 1.5) and it is expected that these components may have different dn/dc values. However, these components cannot be completely separated by Superose column. Each peak includes heterogeneous molecules. It is therefore difficult to use the dn/dc value respectively for each fraction. For this reason, the one common dn/dc valute has been used to evaluate each fraction in the past report of gum Arabic (Al-assaf et al., 2003). Consequently, the molecular weight in this study should be viewed as an apparent value.

The Zimm method constructs a plot of $\check{K}\, c\, /R\, (\theta)$ vs $\sin\hat{}2\ (\theta/2)$ and fits a polynomial in $\sin\hat{}2\ (\theta/2)$ to the data, thereby obtaining Mw and $\langle rg^2 \rangle$ from the intercept and slope at zero angle. It works well for polymer of flexible random coil structure, but it has been observed that the situation changes drastically for large structure (>100nm) and more compact particles as a result of the scattering curve becoming strongly curved particularly in the small angle range. As a result there tends to be over estimation and greater uncertainly of the molecular weight and *Rg* (Al-assaf & Phillip, 2006). Alternatively, Debye fit method constructs a plot of $R(\theta)/(K\hat{} * c)$ vs. $\sin\hat{}2\ \theta/2$ and fit a polynomial in $\sin\hat{}2\ (\theta/2)$ to the data, thereby obtaining M_w and $\langle rg^2 \rangle$ from the intercept and slope at zero angle. It gives

a good result over a wider range of molecular weight as compared to the Zimm formalism. However, the Berry fitting method is most applicable method for large molecules (Burchard, 1994) and therefore used in this study. Berry method constructs a plot of SQRT{$K^{*} c\ /R\ (\theta)$} vs sin^2 ($\theta/2$) and fit a polynomial in sin^2 ($\theta/2$) to the data, thereby obtaining M_w and $\langle rg^2 \rangle$ from the intercept and slope at zero angle. It is very useful for large molecules. Therefor Berry method is used in this study as processing method for molecular weight of gum arabic samples.

3.2.6 Evaluation of emulsification

3.2.6.1 Background

An emulsion is colloidal dispersion system comprises a collection of small particles, in which liquid is dispersed in a continuous liquid phase of different composition, and there is an interfacial layer between the two phases which is seized by some necessary emulsifier. Beverage emulsions are basically prepared to promote the dispersion of non-polar characters of flavor oils and other hydrophobic ingredients, that cannot simply be dispersed directly into an aqueous phase and they would swiftly coalesce and separate leading to a layer of oil on the top of product. Therefore, they first have to be converted into a colloidal dispersion system consisting of flavor oil molecules encapsulated within small particles suspended within an aqueous medium (Daniel, Piorkowski, Julian et al., 2014; McClements & Li, 2007). This colloidal system must be carefully formed and structured to supply desirable physicochemical, sensory and biological attributes to the final product. There are different colloidal delivery systems suitable for beverage emulsion application, with the most common being micro-emulsion, Nano-emulsion and emulsion. Conventional emulsion (r>100 nm) is thermodynamically unstable system, therefor, tend to breakdown during storage through variety of instability mechanisms (see section 1.7 and Figure 1.9).

The classical method of emulsion preparation is, the emulsifier agent firstly dissolved into phase where it is most soluble, after which the second phase is added, thereafter shear is applied to the mixture using either high speed mixing or vigorous agitation. For O/W emulsion the agitation must be turbulent and is crucial to producing sufficiently small droplets (Breuer, 1985). Frequently, after an initial mixing, a later mixing with very high applied mechanical shear forces is required. This second mixing can be provided by a wider range of techniques such as turbine mixer, ultrasound generator and collision generator. It possible then to form a stable emulsion for reasonable period of time by including substance known as stabilizers which include emulsifier (are surface active molecules that adsorb to the surface of freshly formed droplets during homogenization forming a productive layer that prevent the droplets from aggregation), weighting agents (are denes hydrophobic components added to low-density oils to prevent gravitational separation by increasing the density of the oil phase), ripening inhibitors (are water-insoluble component added to polar oil to prevent Ostwald ripening; which is phenomena often found in oil-in-water emulsion, due to instability of the molecules on the surface of particles, therefore the unstable molecules go into solution shrinking particles size, those free molecules deposit on larger particles in order to reach a more thermodynamically stable state. Thus, small particles decreased in size until they disappear, and larger particles grow even larger. This over time causes emulsion instability and eventually phases separation and texture modifies are substance used to increase the viscosity or gel aqueous solution, there by retarding or preventing droplet movement (Daniel et al., 2014). However, there is other factors that can influence the emulsion stability such as, the density difference between the droplet and surrounding fluid; the greater density difference the faster the rate of gravitational separation (McClements, 2004). The refractive index contrast between the droplets and surrounding fluid which determines the efficiency of light scattering by droplets in

emulsion; the greater refractive index contrast between the droplets and surrounding fluid the stronger the degree of light scattering and the more turbid the appearance (McClements, 2002).

Once an emulsion has been formed it is often necessary to characterize the emulsion, specifically in terms of it is average size and it is size distribution. The importance of particle size in determining emulsion stability is evident from Stockes law, Equation 3-21.

$$V = \frac{2r^2(d_2 - d_1)g}{9\mu} \qquad [3\text{-}21]$$

Where: V is velocity of separation (or rate of creaming) (cm/sec), r is droplet radius (cm), d_2 is density of continuous phase, d_1 is density of disperse phase (g/cm^3), g is acceleration of gravity and μ is viscosity of the continuous phase (g/cm.sec).

According to stokes law, the particle size changing will have big effect on the emulsion stability because the velocity of creaming or sedimentation is related to the square of the particle size.

The most direct method to measure emulsion particle size and distribution, which is theoretically least subject to errors, is electron microscopy (Buchhein, 19997). However, this technique cannot be used routinely to determine size distribution. The rapid methods of particle sizing are based on light scattering. Laser diffraction is the most widely used technique for particle size analysis, where four types of interaction occur between light and particle; diffraction in outline of particle (Fraunhofer diffraction, which is evaluation of the wave fields diffracted from plane aperture), reflection on both inside and the outside surfaces of particle, refraction on the interface between particle and dispersion medium, and absorption of light in the particle inside. According to these interactions, the intensity shape of the scattered light is changed due to the particle diameter. Particle size analysis by laser

diffraction is measured by detecting this change. The basic scheme of particle size analysis system is shown in Figure. 3.11.

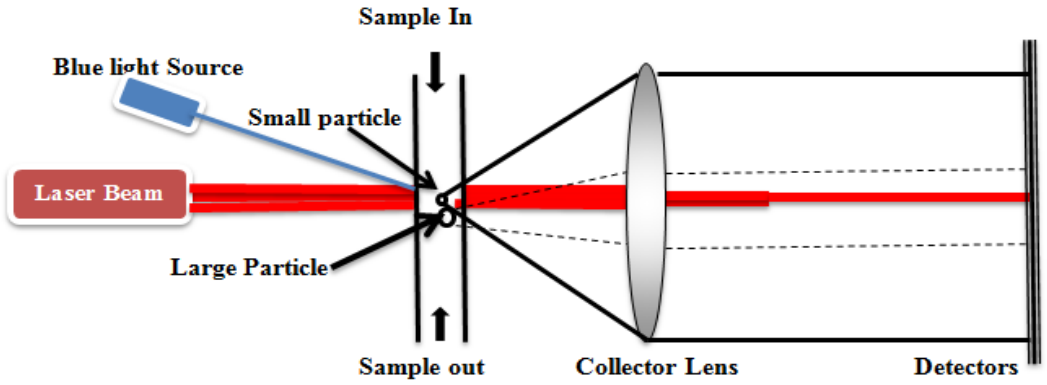


Figure - 3.11. Basic system of laser diffraction for particle size analysis.

The scattered pattern is determined by the relation between the wavelength of light and particle diameter. If the range of particle diameter is from μm to mm, the particle diameter is larger than the wavelength of light, and the light scatter from the edge of the particle at an angle which is dependent on the size of the particle. Larger particles scatter light at relatively smaller angle than light scattered from smaller particles. Moreover, the sample of small particles has more surface area, and the total surface area depends on both the size and shape of particles. However, it is generally inappropriate to characterize dispersion system in terms of a given droplet or particle size. This is because there is inevitably a size distribution and that why the concept of average size it is important.

3.2.6.2 Methods

3.2.6.2.1 Preparation of O/W model beverage emulsion

The test material was used in the kibbled form, dissolved at 30 wt% in distilled water (at room temperature) using a roller mixer overnight, the solution which has insoluble matters

was centrifuged at 2500 rpm for five minutes and subsequently filtered using 100 µm nylon strainer (BD Falcon). Citric acid was added to adjust pH (pH=4), sodium benzoate added as preservative. Crude emulsion premixes were prepared by adding all the components (the additional water to lower gum concentration to 20% w/w). The water phase was prepared by mixing appropriate volume of gum Arabic solution, 10% (w/v) citric acid and 10% (w/v) sodium benzoate according to each formation. The prepared oil phase MCT (Medium Chain Triglyceride) oil was added to the water phase, and the mixed solution was homogenized energetically for 3 minutes by using a bench-top high-speed mixer (a Polytron PT-2100 homogenizer Figure 3.12) at 26000 rpm.

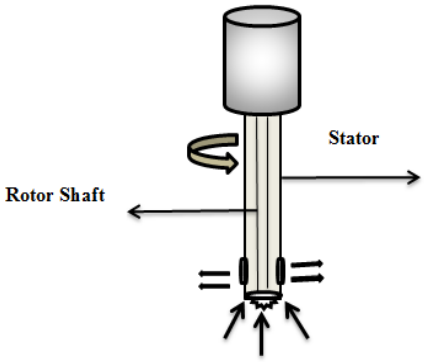


Figure - 3.12. Structure and homogenization mechanism of Polytron. The high-speed rotation of Polytron homogenizer generates a shear stress in the gap between stator part and rotor shaft which causes the large droplets to be broken into smaller ones for making emulsion.

Emulsification (an average droplet size of < 1 µm and narrow particle size distribution) was achieved by subjecting the pre-emulsion to high-pressure valve homogenizer (Nanomizer NM2-L100-D07, Collision type S generator (diameter 84 µm), Yoshida Kikai Co. Ltd. See Figure 3.13) at 50 MPa in two passes. Final fine emulsion divided to two portions, one

portion was kept in a glass vial at 60 C° for accelerated stress testing (Gallenkamp, OVA031. XX1.5) and other part were kept at room temperature (ambient storage).

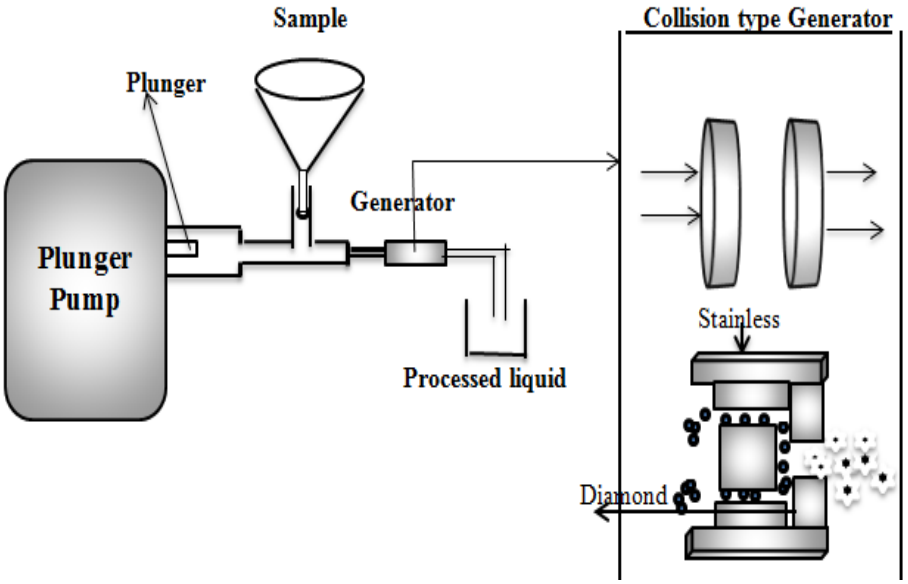


Figure - 3.13. Structure and homogenization mechanism of Nano-Mizer (high pressure homogenizer) gives a combination of intense shear, cavitation and turbulent flow conditions which cause the larger droplets to be broken down into smaller ones.

3.2.6.2.2 Droplet size analysis

Droplet size distribution of the emulsions was analysed using a Mastersizer 2000 laser diffractometer (Malven Instruments, UK). The Mastersizer 2000 measure particle diameters in the range of 0.02 to 2000 µm. Distilled water was used dispersant.

The droplet size of the emulsion was described by the volume median diameter (VMD). The ratio of the particle of 1µm or more and 2 µm or more were also determined.

3.2.6.2.3 Emulsion long-term stability test / Accelerated temperature stress test

The long-term stability of emulsion droplet size is the capability of the emulsion at ambient temperature to remain within it is physical specification and properties that it possessed at

the time of formation; This is an important parameter when the emulsion is used in different applications such as beverage, pharmaceutical etc. The long-term stability test is used to:

provide how the emulsion droplet size varies with time, establish shelf life for the products and determine recommended storage conditions. However, for practical reason the accelerated stress test is often used for investigating the emulsion stability instead of long-term ambient observation.

Tse and Reineccius have reported that elevated temperature give the most accurate prediction of emulsion stability (Tse & Reineccius, 1995). The accelerated stress by heating is generally used instead of the long-term stability test as method of evaluating the emulsion in a short-term. Therefore, the assumption is if the emulsion withstands this stress test, it is to be stable at normal condition of storage.

In this study, the long-term stability of the emulsion for samples of *A. senegal* were evaluated by using the accelerated stress at 60 C°. The spherical particle can be described using a single number the diameter, because every dimension is identical, however, it is important to measure and control the particle size distribution in the emulsion. The particle size for the stored emulsion at 60 C° was measured by laser diffraction a Mastersizer 2000 ‘illustrated in Figure 3.8 at given time intervals (3 days and 7days).

3.2.7 Statistical analysis

3.2.7.1 Background

Statistical methods nowadays are conducted through different types of computing software; one of most simple and widely used statistic software is SPSS, which is the acronym of Statistical Package for the Social Science. SPSS is a comprehensive window based program that can be used for analysing data. SPSS can take data from almost any type of file and use them to generate tabulated reports, charts and plot of distributions and trends descriptive statistics and complex statistical analysis. It is one of the most popular statistical packages which widely used for statistical analysis in field of market research, health research, science, companies survey, government, education and others. In addition to statistical analysis SPSS is also very useful in data management and documentation. SPSS is including different statistics applications in the base software such as, descriptive statistics (mean, standard deviation), bivariate statistics (mean, t-test, ANOVA) and correlation and prediction for numerical outcome (Linear regression) (Kandethody M. Ramachandran. & Tsokos., 2009). In this study samples were analysed by using IBM SPSS statistics version 22, two different type of analysis were applied to obtain the outcome reports and graphs. They were (i) SPSS statistics one-way analysis of variance (ANOVA) combined with the descriptive analysis to calculate the means of the different group and their standard deviation and furthermore to check whether there are significant differences between the means of the group or not and (ii) a linear regression analysis to check the correlations between the means of the different groups and to predicate the numerical outcomes.

3.2.7.2. SPSS for one-way analysis of variance (ANOVA)

The one-way analysis of variance (ANOVA) is an inferential statistical test that used to determine whether there are any significant differences between the means of two or more independent groups.

$$\bar{X} = \frac{\sum_{i=1}^{i=n} X_i}{n} \qquad [3\text{-}22]$$

Where $\bar{X}$the sample is mean, $\sum_{i=1}^{i=n} X_i$ is the sum of the samples and n is the number of the sample observations.

$$\delta = \sqrt{\sum(X_i - \bar{X})^2/(n-1)} \qquad [3\text{-}23]$$

Where δ is standard deviation, X_i is each value of dataset, $\bar{X}$ is mean and n is total number of data points.

ANOVA test the hypothesis that the means of two groups or populations are equal, specifically it tests the null hypothesis.

$$H_\circ : \mu_1 = \mu_2 = \mu_3 = \ldots\ldots\ldots = \mu_k \qquad [3\text{-}24]$$

Where, $H_\circ$ is null hypothesis, μ is group mean and k is number of group. If, however, the one-way ANOVA returns a significant result, the alternative hypothesis (H_A) will accepted, which is that there are at least two group means are significantly different from each other.

ANOVA measure two source of varition in the data and compares their relative size :

Variation between the group $(X1 - X)^2$ and varition within the group $(Xii - Xi)^2$

The ANOVA F- statistic is ratio between groups variation divided by the variation within group. It is (Fixation Indices) term understood in the statistical inference, especially in analysis of varince. Large F-value is evidence indicate that their is more different between groups than within the groupe. From the table of F critical values identify the P- value, it is

area under probability curve, represented the cutoff point for the alpha level of significance variance, usually 0.05. low P-values than 0.05 are strong evidance for significant variations (Marsal, 1987).

One-way ANOVA analysis required appropriate data format to give a valid result. Therefore, part of the process involves checking to make sure that the data subjected to analyse can actually be analysed using a one-way ANOVA, to confirmed that data must passes the following six assumptions that are required for a one-way ANOVA, which are data, should contain dependent variable (should be measured at the interval or ratio level), should also contain independent variable (should consist two or more categorical independent groups), have independence of observations (which means that there is no relationship between the observations in each group or between the groups themselves), there should be no significant outliers, dependent variable should be approximately normally distributed for each category of the independent variable, and there need to be homogeneity of variances.

It is important to realize that one-way ANOVA analysis is an omnibus test statistic and cannot tell which specific groups are significantly different from each other, it only used to tell at least two groups are different, since there may three, four, five or more groups depend on the study design. Thus, to determine which of these groups differ from each other is important therefore; need to use a post-hoc test. The word post-hoc is from Latin which mean 'after this', in practice post-hoc test usually concerned with finding patterns and /or relationship between subgroups of samples that would otherwise remain undetected and discovered. Post-hoc test is designed for situation in which the analysis of (ANOVA) has already obtained a significant omnibus F-test with a factor that consists of three or more means and additional exploration of the differences a mong means is needed to provide specific information on which means are significantly different from each other.

3.2.7.3 SPSS correlation and regression linear analysis

3.2.7.3.1 Correlation

Correlation is statistical analysis which measure and analysis the degree or extent to which the two variables fluctuate with refernce to each other; is attempt to determine the degree of relationship between variables or analysis the covariation between two or more variables. In correlation analysis is very important to find the nature and degree of the relationship between the two variables. Correlation is classified into various type such as, postive and negative, linear and non-linear, partial and total, and simple and multiple. The computation of correlation is to pointout the correlation coefficient, which denoted by (r). The covariation between the variables X and Y is defined as

$$\text{Cov}\,(X,Y) = \sum \frac{(x-\bar{x})(y-\bar{y})}{n} \qquad [3\text{-}25]$$

Where$\bar{x}$, $\bar{y}$ are respectively means of X and Y and n is the number of pairs of observation. Karl pearson's coefficient of correlation is statistician suggest a mathematical method for measuring the magnitude of linear relationship between the two variables. It is most widely used method in practice, it denoted by r^2.

The correlation coefficient r values lies between -1 and +1, and the interpretation of the r values is, if r = +1 is indicate to perfect positive correlation, if r = -1 is indicate to perfect negative correlation, if r = 0 is indicate to no relationship between the variables and if r value near to zero is indicate to less degree of correlation (Liu, Kuang, Gong et al., 2003).

3.2.7.3.2 Regression

Regression analysis classified into simple and multiple, linear and non-linear, and total and partial. The term linear is used because equations (3-26 and 3-27) are linera function of the unknown parameters b_0, b_1, b_2,..... b_p. The linear regressions are based on stright-

line and normaly it is taken into account because it has batter predective value and also show the average relationship (correlation) between two or more variables.

$$Y = a + b_1x_1 + b_2x_2 + \ldots\ldots + b_px_p \qquad [3\text{-}26]$$

$$X = a + b_1y_1 + b_2y_2 + \ldots\ldots + b_py_p \qquad [3\text{-}27]$$

Where a and b are the regression coefficients, a is often called the constant of the intercept, while b is called variable regression coefficient because it determines how the predicted the other variable values. From the regression equations (3-19 and 3-20) the normal equastions are

$$\sum XY = a\sum Y + b\sum Y^2 \qquad [3\text{-}28]$$

$$\sum YX = a\sum X + b\sum X^2 \qquad [3\text{-}29]$$

From the normal equations, the coefficient regression constant a and b can be calculated. The linear correlation coefficient denoted by r, which measured the strength and the direction of a linear relationship between two variables; and sometimes statistically referred to Pearson product moment correlation coefficient in honour of its developer Karl Pearson.

$$r = \frac{n\sum XY - (\sum X)(\sum y)}{\sqrt{n(\sum X^2) - (\sum X)^2}\sqrt{n(\sum Y^2) - (\sum Y)^2}} \qquad [3\text{-}30]$$

Where n is the number of pairs of data.

The coefficient of determination which statistically denoted by r^2 is useful coefficient because it gives the proportion of the variance (fluctuation) of one variable that is predictable from the other variables. r^2 Is used as the ratio of the explained variation to the total variation: the values r^2 are $0 \leq r^2 \leq 1$. It allowed to determine how certain can

make predication from certain statistic model or graph (Iserbyt, Schouppe, & Charlier, 2015).

Multiple linear regression analysis is used to establish the statistical relations between one dependent variable Y and one or more independent variables x_1, x_2,..... x_p.

In many situation in the linear regression analysis we need to deal with independent categorical variables and regression analysis treats all independent variables in the analysis as numerical. Therefore, a dummy variable is created in this situation to trick the regression algorithm into correctly analysing attribute variables. It is useful way of coding to change the qualitative variables to quantitively variables and it makes it easier to turn the model into a decision tool. A dummy variable is only takes the value 0 or 1 to indicated the absence or presence of some categorical effect that may expected to shift the outcome (Golden & Richard, 1996).

D =1 if the criterion is satisfied.

D= 0 if not.

As a rule should always include one less dummy variable in the model than there categorical variables, otherwise will introduce multicollinearity into the model.

D = k-1

Where, D = dummy variables and k = categorical variables

The simple regression equation is

$$y = a + b_1 x_1 \qquad [3\text{-}31]$$

where, y = dependent variable, a = constant equal the intercept, b = constant equal the slop and x = independent variable.

when using a dummy independent variable coding D to represent the categorical independent variables.

$$y = a + b_1 D_1 \qquad [3\text{-}32]$$

because a dummy variable takes only the value 1 or 0 then

when D =1

$$y = a + b \qquad [3\text{-}33]$$

and when D = 0

$$y = a \qquad [3\text{-}34]$$

The different values of y in the two-above equation showed the different between two categoricals groups shown in Figure 3.14.

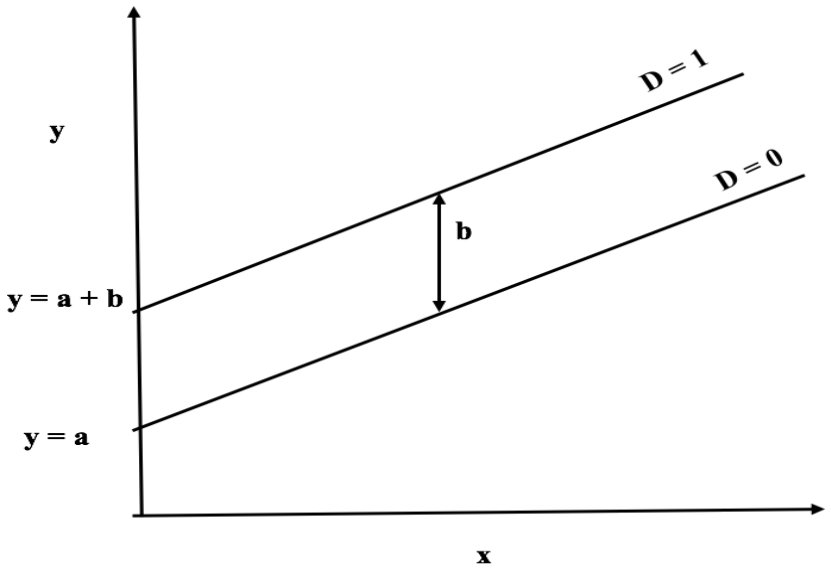
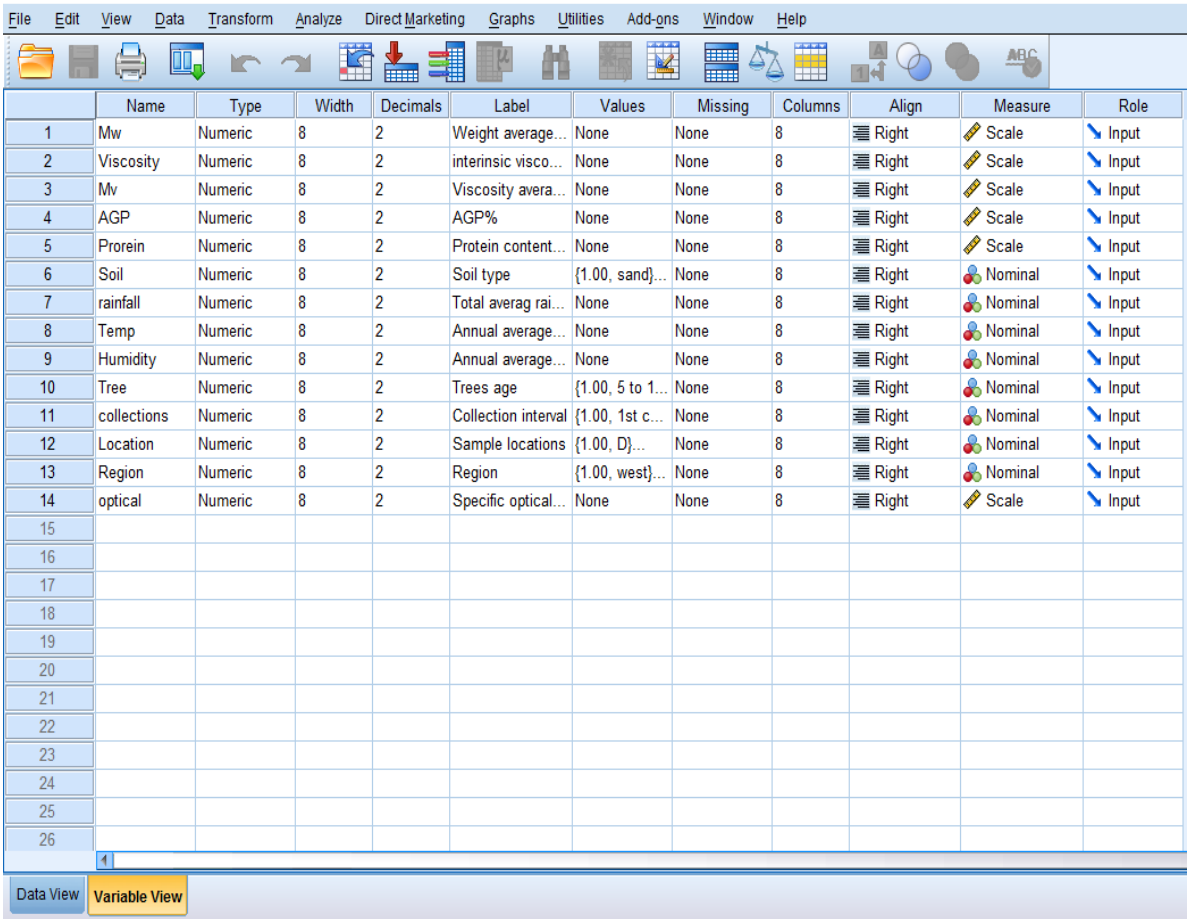


Figure - 3.14. Measure average difference between two categorical groups using a dummy variables coding.

3.2.7.4. SPSS analysis procedure

Both ANOVA analysis, correlation and linear regression analysis could be applied simultaneously in SPSS by using the same entered data. When the six assumptions in the previous section (3.2.7.2) have not been violated, then the data will have imported to the SPSS by using two different windows; variable window which contain full description of the inserted variable such as (name, type, width, label, value) shown in Figure 3.15, and data view window which contain the value of all entered variables and where the data could be imported, shown in Figure 3.16.



	Name	Type	Width	Decimals	Label	Values	Missing	Columns	Align	Measure	Role
1	Mw	Numeric	8	2	Weight average...	None	None	8	Right	Scale	Input
2	Viscosity	Numeric	8	2	interinsic visco...	None	None	8	Right	Scale	Input
3	Mv	Numeric	8	2	Viscosity avera...	None	None	8	Right	Scale	Input
4	AGP	Numeric	8	2	AGP%	None	None	8	Right	Scale	Input
5	Prorein	Numeric	8	2	Protein content...	None	None	8	Right	Scale	Input
6	Soil	Numeric	8	2	Soil type	{1.00, sand}...	None	8	Right	Nominal	Input
7	rainfall	Numeric	8	2	Total averag rai...	None	None	8	Right	Nominal	Input
8	Temp	Numeric	8	2	Annual average...	None	None	8	Right	Nominal	Input
9	Humidity	Numeric	8	2	Annual average...	None	None	8	Right	Nominal	Input
10	Tree	Numeric	8	2	Trees age	{1.00, 5 to 1...	None	8	Right	Nominal	Input
11	collections	Numeric	8	2	Collection interval	{1.00, 1st c...	None	8	Right	Nominal	Input
12	Location	Numeric	8	2	Sample locations	{1.00, D}...	None	8	Right	Nominal	Input
13	Region	Numeric	8	2	Region	{1.00, west}...	None	8	Right	Nominal	Input
14	optical	Numeric	8	2	Specific optical...	None	None	8	Right	Scale	Input
15											
16											
17											
18											
19											
20											
21											
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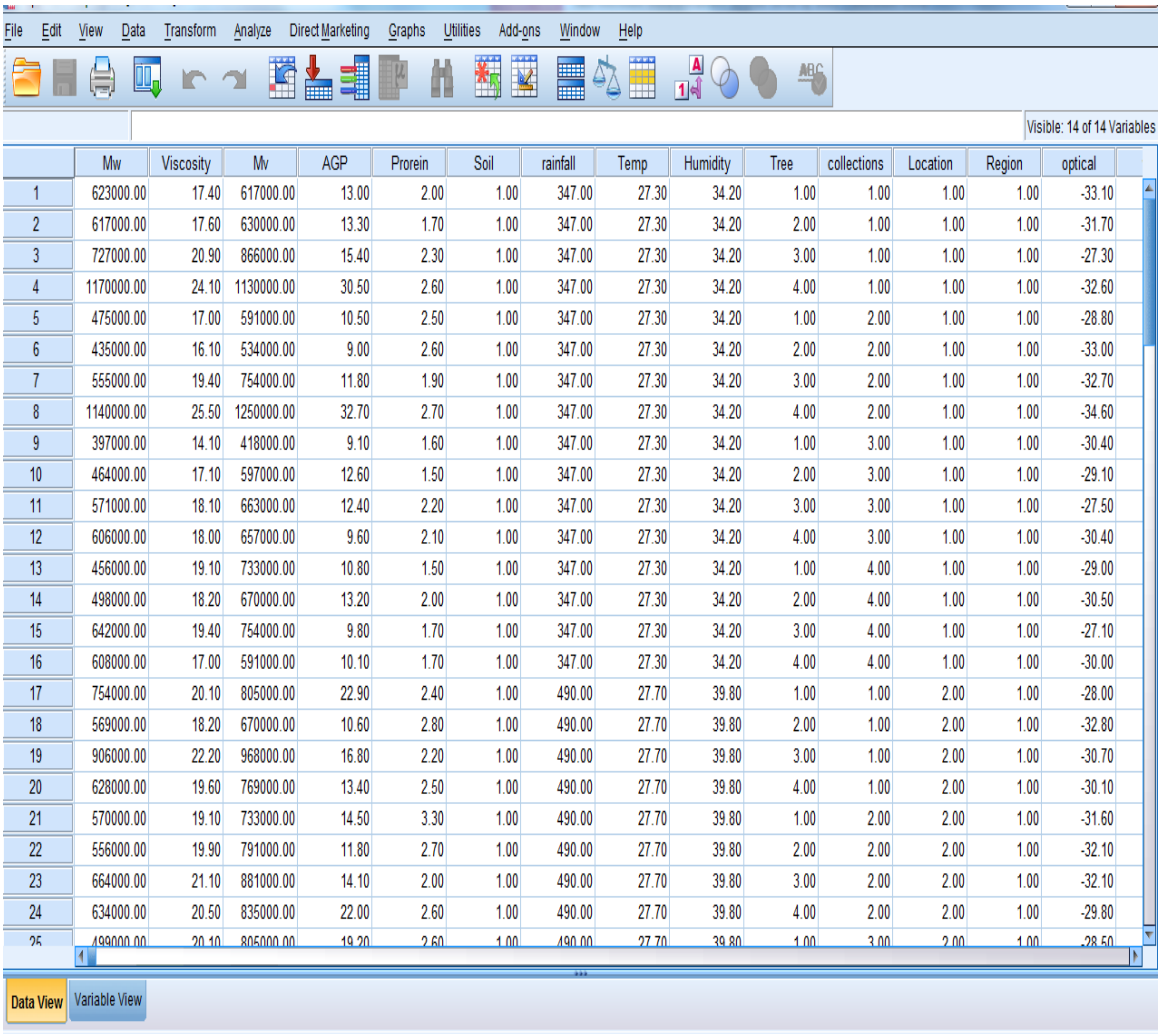
23											
24											
25											
26											

Figure - 3.15. Variable view window showing the name, label, measure and value for dependent and independent variables.



	Mw	Viscosity	Mv	AGP	Prorein	Soil	rainfall	Temp	Humidity	Tree	collections	Location	Region	optical
1	623000.00	17.40	617000.00	13.00	2.00	1.00	347.00	27.30	34.20	1.00	1.00	1.00	1.00	-33.10
2	617000.00	17.60	630000.00	13.30	1.70	1.00	347.00	27.30	34.20	2.00	1.00	1.00	1.00	-31.70
3	727000.00	20.90	866000.00	15.40	2.30	1.00	347.00	27.30	34.20	3.00	1.00	1.00	1.00	-27.30
4	1170000.00	24.10	1130000.00	30.50	2.60	1.00	347.00	27.30	34.20	4.00	1.00	1.00	1.00	-32.60
5	475000.00	17.00	591000.00	10.50	2.50	1.00	347.00	27.30	34.20	1.00	2.00	1.00	1.00	-28.80
6	435000.00	16.10	534000.00	9.00	2.60	1.00	347.00	27.30	34.20	2.00	2.00	1.00	1.00	-33.00
7	555000.00	19.40	754000.00	11.80	1.90	1.00	347.00	27.30	34.20	3.00	2.00	1.00	1.00	-32.70
8	1140000.00	25.50	1250000.00	32.70	2.70	1.00	347.00	27.30	34.20	4.00	2.00	1.00	1.00	-34.60
9	397000.00	14.10	418000.00	9.10	1.60	1.00	347.00	27.30	34.20	1.00	3.00	1.00	1.00	-30.40
10	464000.00	17.10	597000.00	12.60	1.50	1.00	347.00	27.30	34.20	2.00	3.00	1.00	1.00	-29.10
11	571000.00	18.10	663000.00	12.40	2.20	1.00	347.00	27.30	34.20	3.00	3.00	1.00	1.00	-27.50
12	606000.00	18.00	657000.00	9.60	2.10	1.00	347.00	27.30	34.20	4.00	3.00	1.00	1.00	-30.40
13	456000.00	19.10	733000.00	10.80	1.50	1.00	347.00	27.30	34.20	1.00	4.00	1.00	1.00	-29.00
14	498000.00	18.20	670000.00	13.20	2.00	1.00	347.00	27.30	34.20	2.00	4.00	1.00	1.00	-30.50
15	642000.00	19.40	754000.00	9.80	1.70	1.00	347.00	27.30	34.20	3.00	4.00	1.00	1.00	-27.10
16	608000.00	17.00	591000.00	10.10	1.70	1.00	347.00	27.30	34.20	4.00	4.00	1.00	1.00	-30.00
17	754000.00	20.10	805000.00	22.90	2.40	1.00	490.00	27.70	39.80	1.00	1.00	2.00	1.00	-28.00
18	569000.00	18.20	670000.00	10.60	2.80	1.00	490.00	27.70	39.80	2.00	1.00	2.00	1.00	-32.80
19	906000.00	22.20	968000.00	16.80	2.20	1.00	490.00	27.70	39.80	3.00	1.00	2.00	1.00	-30.70
20	628000.00	19.60	769000.00	13.40	2.50	1.00	490.00	27.70	39.80	4.00	1.00	2.00	1.00	-30.10
21	570000.00	19.10	733000.00	14.50	3.30	1.00	490.00	27.70	39.80	1.00	2.00	2.00	1.00	-31.60
22	556000.00	19.90	791000.00	11.80	2.70	1.00	490.00	27.70	39.80	2.00	2.00	2.00	1.00	-32.10
23	664000.00	21.10	881000.00	14.10	2.00	1.00	490.00	27.70	39.80	3.00	2.00	2.00	1.00	-32.10
24	634000.00	20.50	835000.00	22.00	2.60	1.00	490.00	27.70	39.80	4.00	2.00	2.00	1.00	-29.80
25	498000.00	20.10	805000.00	19.20	2.60	1.00	490.00	27.70	39.80	1.00	3.00	2.00	1.00	-28.50

Figure - 3.16. Data view window. Where the data to be imported for the analysis.

To apply ANOVA, analysis or correlation and linear regression analysis in the SPSS; on the top menu of data view window which shown in figure 3.16, for ANOVA analysis, click Analyse > Compare Mean > One-way ANOVA as illustrated in Figure 3.17.

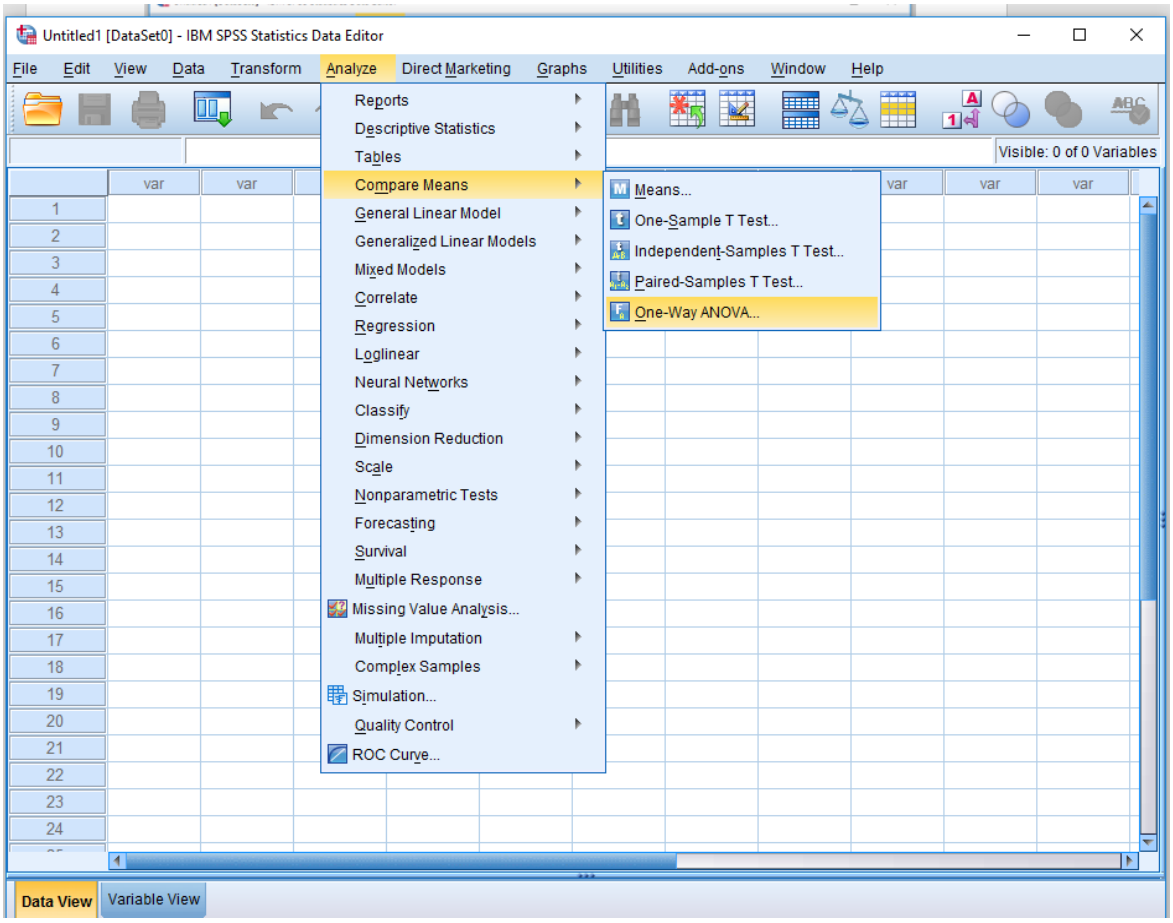


Figure - 3.17. Analysis window which contains different type of analysis options such as One-Way ANOVA, correlation, linear regression analysis, ….ect

For correlation analysis from the same window of data view on the top menu click Analyse > correlate > Bivariate as shown in Figure 3.16.

Chapter 4

4. Characterisation of *A. senegal* samples

In this chapter, the 168 samples will be evaluated using the methods described in the previous chapter such as moisture content, optical rotation, protein content, intrinsic viscosity, molecular weight and molecular weight distribution. The results are tabulated in Table 4.1 and discussed in turn below.

4.1 Results and discussion

Values between 10.5% - 15% were obtained for the % loss on drying (moisture content) as shown in Table 4-1. These values were in agreement with previously reported studies (Anderson, Hirst, & Rahman, 1967; Idris et al., 1998; K. Karamalla et al., 1998). Table 4-1 also gives the values of the specific optical rotation which were found to be between -25.3 to -36.3. The results were also in good agreement with the values reported previously and confirm the average optical rotation value (~-30) associated with *A. senegal* var. *senegal* (Anderson et al., 1967; GAC, 2009; Idris et al., 1998; K. Karamalla et al., 1998; Vandevelde & Fenyo, 1985).

The protein content was measured by using Lowry method (see section 3.2.2) and results are also tabulated in Table 4.1. Values between 1.5 – 3.3% with an average of ~2% were obtained which are again typical values reported previously for *A. senegal var. senegal* variety (Al-Assaf et al., 2005; Idris et al., 1998).

The intrinsic viscosity was determined as described in section (3.2.4) and the results are tabulated also in Table 4.1. Values in the range of 17.8 g/cm3 – 22. 8 cm^3/g were obtained and in good agreement with previously reported values for *A. senegal* var. *senegal* (Anderson et al., 1967; Duvallet et al., 1993; GAC, 2009; K. Karamalla et al., 1998).

The viscosity average molecule weight results (M_v) were also listed in Table 4-1 calculated according to "Mark-Houwink" equation (see section 3.2.4.1). The values of the viscosity average molecule weight were between $4.45 \times 10^5 - 9.49 \times 10^5$ g/mol. The results of intrinsic viscosity and viscosity average molecular weight demonstrate the natural built in variation present in Gum arabic as shown in Figures 4.1 and 4.2 respectively.

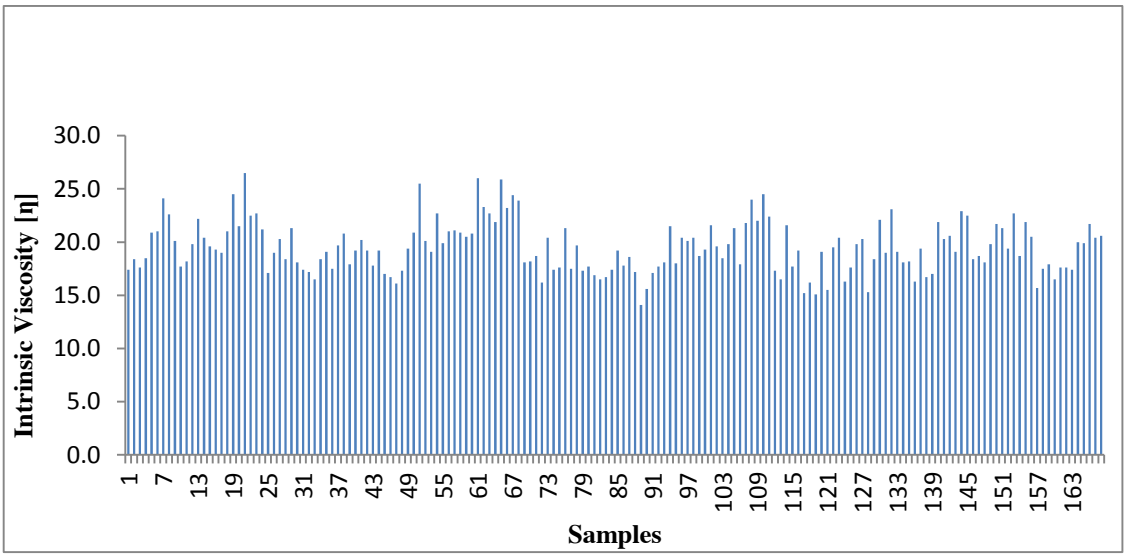


Figure - 4.1. Intrinsic viscosity [η] for the all fresh samples used in this study.

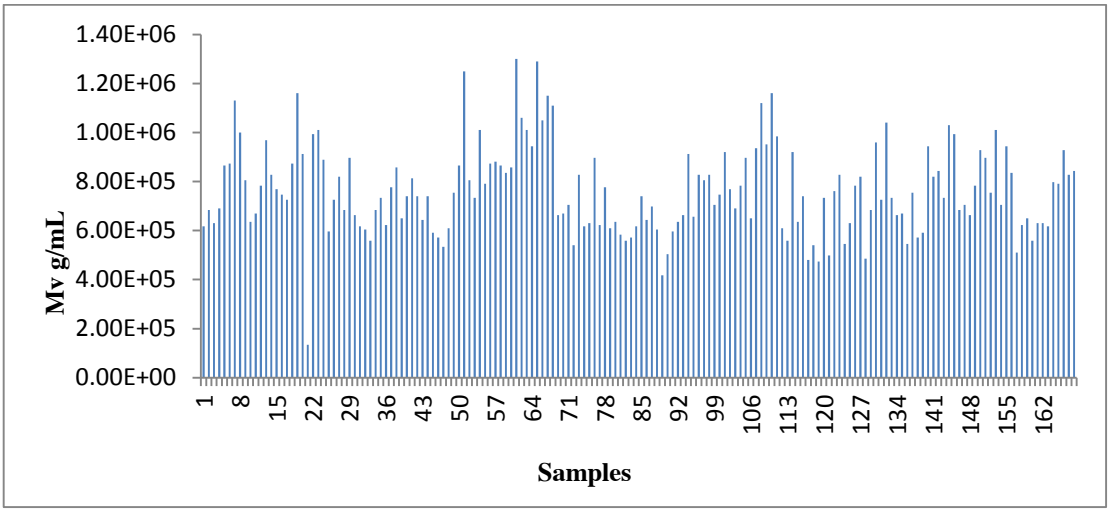


Figure - 4.2. Viscosity average molecular molecule weight for all the fresh samples used in this study.

Figures 4.3, 4.4 and 4.5 show typical elution profile of three gum arabic samples of high, medium and low molecular weight respectively. The elution profile, as described in section (3.2.5), obtained using GPC coupled with three detection system, namely: a multi angel laser light scattering (MALLS), refractive index (RI), and UV. All three samples gave typical response associated with *A. senegal* gum as described below. The light scattering response at detector 90^{o}, a measure of concentration and mass, shows the presence of two peaks. The first peak has a high response since it is associated with the high molecular weight material termed arabinogalactan – protein fraction (AGP). The second peak is broader with lower response and it accounts for the rest of the gum, arabinogalactan (AG) and Glycoprotein (GP). The RI response, a measure of concentration, shows the presence of two peaks also but the response is opposite to that in MALLS detector. The opposite peak height response obtained from the two detectors can be explained purely based on the molecular weight of the eluted fraction. The AGP is approximately around 10% of the total of the gum but with higher molecular weight compared to the AG and hence shows higher response on the light scattering detector whereas the AG (90% of the gum) shows higher response on the RI detector.

The UV detector response shows the response of three peaks. The first peak is associated with the AGP fraction which has the protein core and carbohydrates units attached to it. The second peak appears as a shoulder immediately after the AGP and corresponds to the AG. Finally, the third peak elutes before the total volume and corresponds to the GP peak. The GP peak is not detected on the light scattering detector since it has low molecular weight and cannot be distinguished in terms of mass from the AG peak. Also, it cannot be seen on the RI because it is detected as a concentration similar to the AG.

The results given above are consistent with previous studies on gum arabic (*A. senegal* var. *senegal*) where the existence of the three main fractions: Arabinogalactan–protein (AGP),

Arabinogalactan (AG) and Glycoprotein (GP) can be clearly identified. (Al-assaf et al., 2003; Churms et al., 1983; Idris et al., 1998; R. C Randall et al., 1989; Williams & Langdon, 1995). Table 4.1 also lists the value of the weight average molecular weight of the whole gum and high molecular weight fraction (% AGP) of the total gum. The weight average molecular weight of the whole gum varied from 3.05 x 10^5 – 1.42 x 10^6 g/mol and in good agreement with values reported previously (Al-assaf et al., 2003; Churms et al., 1983; Idris et al., 1998; R. C Randall et al., 1989; Williams & Langdon, 1995). The proportion of the AGP component was in the range (5.3% - 32.0%) of the total gum. Some of these values are higher than the range of 5 -15% reported previously in the literature (Al-assaf et al., 2003; Churms et al., 1983; Idris et al., 1998; R. C Randall et al., 1989; Williams & Langdon, 1995).

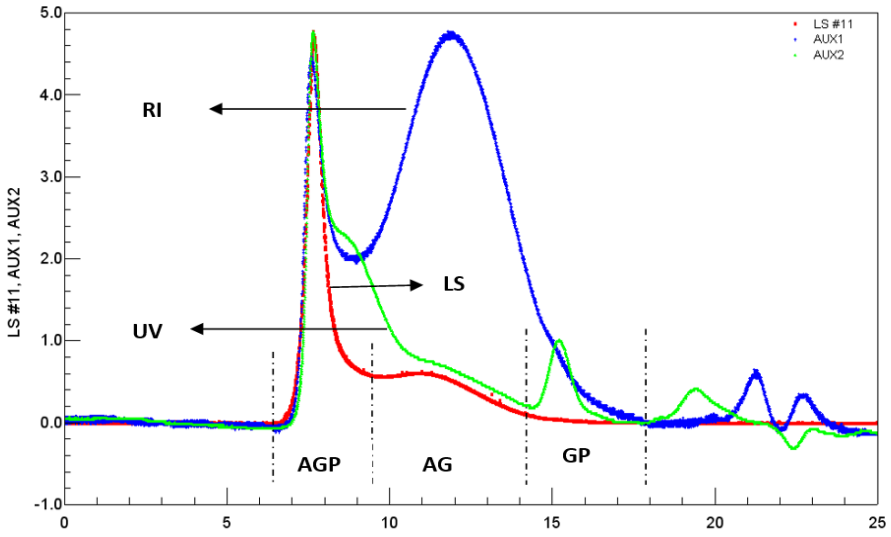


Figure - 4.3. Typical elution profile of high molecular weight *A. senegal* sample (TT15P1) obtained by using GPC –MALLS. Red line is detector 90°, blue line is the refractive index and green line is the UV detector at 214nm.

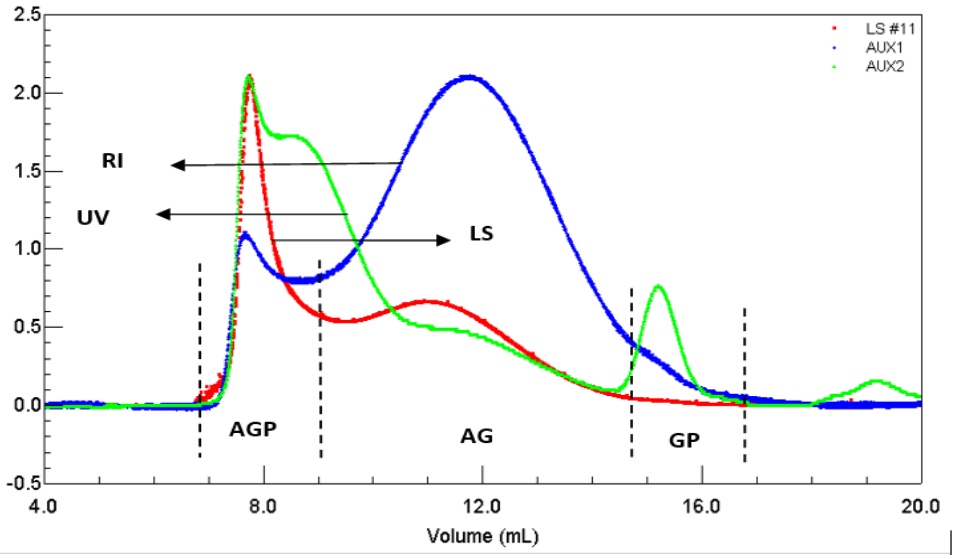


Figure - 4.4. Typical elution profile of medium molecular weight *A. senegal* sample (BT10P1) obtained by using GPC –MALLS. Red line is detector 90º, blue line is the refractive index and green line is the UV detector at 214nm.

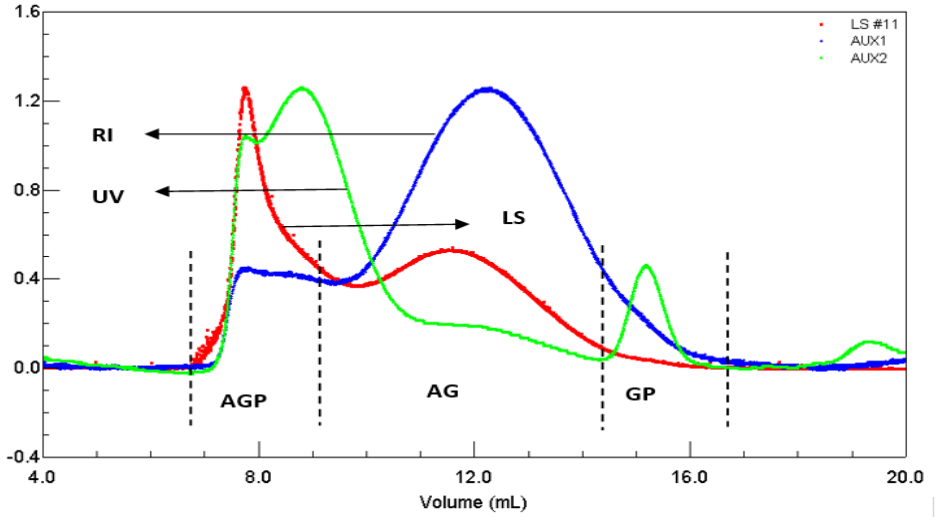


Figure - 4.5. Typical elution profile of low molecular weight *A. senegal* sample (KR10P2) obtained by using GPC –MALLS. Red line is detector 90º, blue line is the refractive index and green line is the UV detector at 214nm.

The results given above clearly show that GPC-MALLS is a powerful technique which allows the determination of the weight average molecular weight for the whole gum as well the proportion of individual fractions. Both values of the average molecular weight and high molecular weight fraction percentage (%AGP) exhibited the natural inherent built in

variation commonly associated with hydrocolloids such as Gum arabic shown in Figures 4.6 and 4.7 respectively.

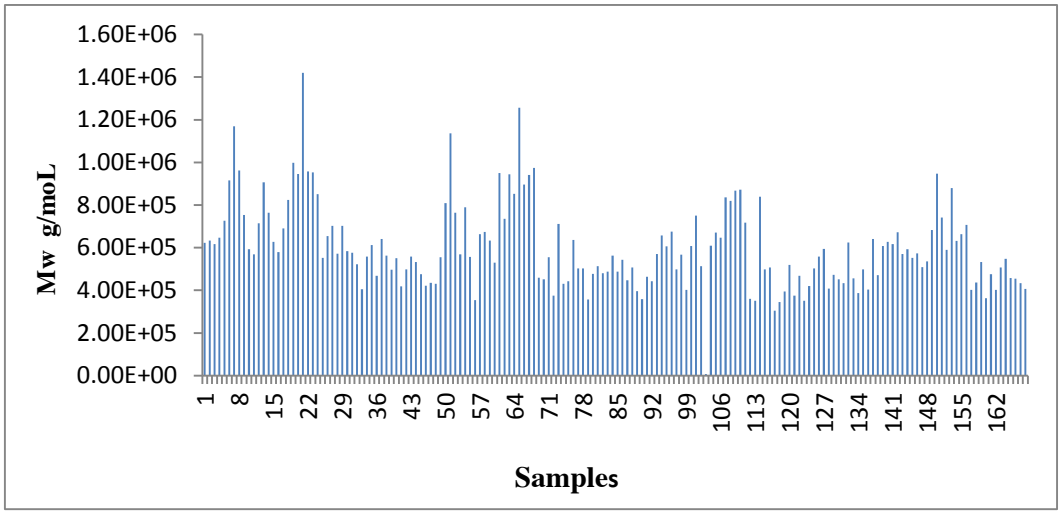


Figure - 4.6. Weight average molecule weight for all the fresh samples used in this study.

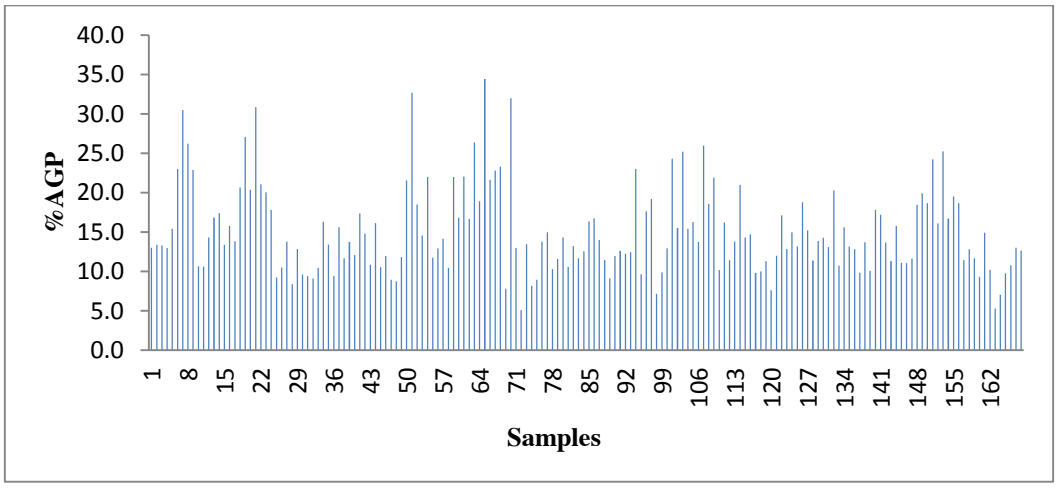


Figure - 4.7. %AGP for all the fresh samples used in this study.

The results of the weight average molecular weight showed very close similarity with the result of viscosity average molecular weight calculated from intrinsic viscosity in terms of trend. The value of viscosity average molecular weight (Mv) is tend to be slightly higher

than the value of weight average molecular weight (Mw), however, there is no significant variation between two values which illustrated in Figure 4.8.

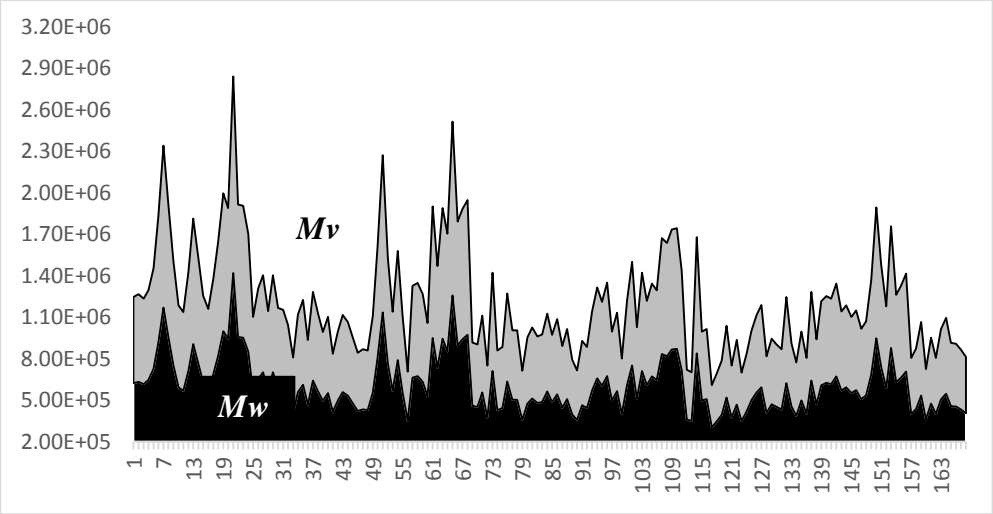


Figure - 4.8. Comparison of M_w and M_v for the all fresh samples.

4.2 Conclusion

The overall conclusions of this chapter are as follows:

- All samples are typical *A*. *senegal* var. *senegal* as determined by the optical rotation, protein content and molecular weight elution profile obtained by GPC-MALLS.
- The natural built in variation commonly associated with hydrocolloids such as Gum arabic is clearly demonstrated through the various parameters measured on all samples.
- There is a clear difference between samples even within the same location

Furthermore, the results showed very good agreement with previous studies and also exhibited the natural inherent variation widely reported for Gum arabic. All the tested samples showed the typical elution profile of *A*. *senegal* gum with the main three distinctive fractions Arabinogalactan – protein (AGP), Arabinogalactan (AG) and Glycoprotein (GP). However, in molecular weight distribution the concentration of the high molecular fraction

(% AGP) is higher than the value reported previously in the literature, that could be explain due to limited number of samples that examined by the all previous studies, in compare to this study which covered broader area in gum arabic belt in Sudan and higher number of samples.

All results listed in Table 4-1 and discussed in (section 4.1) will be analysed in chapter 5 by using SPSS statistics one-way analysis of variance (ANOVA), correlation and linear regression analysis.

Table 4-1. Result of the fresh samples.

S.NO	Sample code	Region	Plantation code	Moisture %	Optical rotation	Protein content (%wt)	Intrinsic viscosity (cm^3/g)	M_v E+5	M_w E+5	AGP (%wt)
1-	DT5P1	West	D	13.2	-33.1	2.0	17.4	6.17	6.23	13.0
2-	DR5P1	West	D	13.0	-33.0	2.1	18.4	6.84	6.34	13.4
3-	DT11P1	West	D	13.3	-31.7	1.7	17.6	6.3	6.17	13.3
4-	DR11P1	West	D	13.1	-33.1	1.9	18.5	6.91	6.47	13.0
5-	DT16P1	West	D	13.1	-27.3	2.3	20.9	8.66	7.27	15.4
6-	DR16P1	West	D	11.3	-31.0	2.5	21	8.73	9.15	23.0
7-	DT20P1	West	D	11.8	-32.6	2.6	24.1	11.3	11.77	30.5
8-	DR2OP1	West	D	11.9	-32.6	2.6	22.6	10.0	9.60	26.2
9-	NT5P1	West	N	13.3	-28.8	2.4	20.1	8.05	7.54	22.9
10-	NR5P1	West	N	13.3	-30.3	2.1	17.7	6.36	5.93	10.7
11-	NT11P1	West	N	12.9	-33.0	2.8	18.2	6.7	5.69	10.6
12-	NR11P1	West	N	11.5	-29.7	2.3	19.8	7.83	7.15	14.3
13-	NT16P1	West	N	12.2	-32.7	2.2	22.2	9.68	9.06	16.8

14-	NR16P1	West	N	12.9	-29.4	2.7	20.4	8.28	7.64	17.4
15-	NT20P1	West	N	13.3	-34.6	2.5	19.6	7.69	6.28	13.4
16-	NR20P1	West	N	13.4	-32.4	2.4	19.3	7.47	5.80	15.8
17-	TT5P1	West	T	13.6	-30.4	2.3	19.0	7.26	6.91	13.8
18	TR5P1	West	T	13.6	-27.5	2.3	21.0	8.73	8.24	20.7
19-	TT11P1	West	T	14.2	-29.1	2.2	24.5	11.6	9.98	27.1
20-	TR11P1	West	T	13.9	-29.0	2.4	21.5	9.12	9.45	20.4
21-	TT16P1	West	T	13.6	-27.5	2.3	26.5	1.34	14.2	30.9
22-	TR16P1	West	T	14.2	-32.0	2.7	22.5	9.93	9.57	21.1
23-	TT2OP1	West	T	13.7	-30.4	2.1	22.7	10.1	9.53	20.0
24-	TR20P1	West	T	15.0	-31.6	2.1	21.2	8.89	8.51	17.8
25-	KT5P1	East	Kh	13.7	-29.0	1.8	17.1	5.97	5.52	9.20
26-	KR5P1	East	Kh	13.9	-29.0	1.9	19	7.26	6.54	10.5
27-	KT11P1	East	Kh	13.9	-30.5	1.8	20.3	8.2	7.02	13.8
28-	KR11P1	East	Kh	13.0	-28.7	1.9	18.4	6.84	5.72	8.40
29-	KT16P1	East	Kh	12.3	-27.1	1.9	21.3	8.97	7.02	12.8

30-	KR16P1	East	Kh	10.5	-31.3	1.9	18.1	6.63	5.80	9.60
31-	KT20P1	East	Kh	12.4	-30.0	2.0	17.4	6.17	5.77	9.40
32-	KR20P1	East	Kh	12.5	-32.1	2.1	17.2	6.04	5.22	9.10
33-	BT5P1	East	B	12.8	-28.0	2.0	16.5	5.59	4.05	10.5
34-	BR5P1	East	B	12.2	-31.3	2.1	18.4	6.84	5.59	16.3
35-	BT11P1	East	B	12.5	-32.8	2.4	19.1	7.33	6.13	13.4
36-	BR11P1	East	B	12.5	-30.7	2.0	17.5	6.23	4.68	9.40
37-	BT16P1	East	B	14.4	-30.7	2.3	19.7	7.76	6.41	15.6
38-	BR16P1	East	B	13.6	-27.5	2.2	20.8	8.58	5.63	11.7
39-	BT20P1	East	B	12.9	-30.1	2.0	17.9	6.5	4.97	13.7
40-	BR20P1	East	B	12.8	-31.5	1.9	19.2	7.4	5.51	12.1
41-	GT5P1	East	G	12.9	-31.6	2.5	20.2	8.13	4.19	17.4
42-	GR5P1	East	G	12.6	-28.6	2.5	19.2	7.4	4.99	14.8
43-	GT11P1	East	G	14.3	-32.1	2.6	17.8	6.43	5.59	10.9
44-	GR11P1	East	G	14.4	-29.2	2.4	19.2	7.4	5.33	16.1
45-	DT5P2	West	D	14.2	-32.1	2.5	17	5.91	4.75	10.5

46-	DR5P2	West	D	11.9	-31.1	2.1	16.7	5.71	4.22	12.0
47-	DT11P2	West	D	13.3	-29.8	2.6	16.1	5.34	4.35	9.0
48-	DR11P2	West	D	12.6	-36.3	4.6	17.3	6.1	4.30	8.80
49-	DT16P2	West	D	12.0	-28.5	1.9	19.4	7.54	5.55	11.8
50-	DR16P2	West	D	12.2	-29.7	2.7	20.9	8.66	8.09	21.5
51-	DT20P2	West	D	13.8	-30.3	2.7	25.5	12.5	11.40	32.7
52-	DR20P2	West	D	13.0	-31.2	2.9	20.1	8.05	7.65	18.5
53-	NT5P2	West	N	13.3	-27.9	3.3	19.1	7.33	5.70	14.5
54-	NR5P2	West	N	13.0	-28.0	2.2	22.7	10.1	7.90	22.0
55-	NT11P2	West	N	13.6	-36.3	2.7	19.9	7.91	5.56	11.8
56-	NR11P2	West	N	13.0	-31.4	1.9	21	8.73	3.54	12.9
57-	NT16P2	West	N	13.4	-34.0	2.0	21.1	8.81	6.64	14.1
58-	NR16P2	West	N	13.4	-31.5	1.9	20.9	8.66	6.75	10.4
59	NT20P2	West	N	13.2	-30.7	2.6	20.5	8.35	6.34	20.5
60	NR20P2	West	N	12.0	-31.0	2.3	20.8	8.58	5.30	20.8
61	TT5P2	West	T	12.3	-29.9	2.3	26	13.0	9.50	26

62	TR5P2	West	T	12.3	-31.0	2.7	23.3	10.6	7.35	23.3
63	TT11P2	West	T	11.7	-28.0	2.0	22.7	10.1	9.45	22.7
64	TR11P2	West	T	11.8	-30.8	2.4	21.9	9.44	8.52	21.9
65	TT16P2	West	T	11.4	-27.4	2.3	25.9	12.9	12.6	25.9
66	TR16P2	West	T	12.2	-29.6	2.1	23.2	10.5	8.96	23.2
67	TT20P2	West	T	11.5	-30.7	2.9	24.4	11.5	9.41	24.4
68	TR20P2	West	T	12.1	-34.9	2.6	23.9	11.1	9.74	23.9
69	KT5P2	East	Kh	11.8	-25.3	2.0	18.1	6.63	4.59	18.1
70	KR5P2	East	Kh	11.8	-26.7	2.2	18.2	6.7	4.52	18.2
71	KT11P2	East	Kh	11.5	-27.5	2.1	18.7	7.05	5.56	18.7
72	KR11P2	East	Kh	12.7	-27.1	2.5	16.2	5.4	3.76	16.2
73	KT16P2	East	Kh	12.0	-26.3	2.1	20.4	8.28	7.11	20.4
74	KR16P2	East	Kh	11.8	-26.9	1.9	17.4	6.17	4.31	17.4
75	KT20P2	East	Kh	12.3	-32.6	1.9	17.6	6.3	4.43	17.6
76	KR20P2	East	Kh	14.9	-34.5	2.1	21.3	8.97	6.36	21.3
77	BT5P2	East	B	13.7	-29.0	2.4	17.5	6.23	5.03	17.5

78	BR5P2	East	B	12.2	-30.6	2.1	19.7	7.76	5.03	19.7
79	BT11P2	East	B	11.4	-26.9	1.7	17.3	6.1	3.57	17.3
80	BR11P2	East	B	11.4	-28.8	2.0	17.7	6.36	4.77	17.7
81	BT16P2	East	B	11.7	-29.7	2.2	16.9	5.84	5.13	16.9
82	BR16P2	East	B	10.9	-28.7	2.2	16.5	5.59	4.80	16.5
83	BT20P2	East	B	11.8	-29.7	2.4	16.7	5.71	4.88	16.7
84	BR20P2	East	B	11.8	-31.9	2.6	17.4	6.17	5.63	17.4
85	GT5P2	East	G	12.9	-30.1	1.9	19.2	7.4	4.87	19.2
86	GR5P2	East	G	11.5	-29.5	1.9	17.8	6.43	5.43	17.8
87	GT11P2	East	G	13.2	-28.8	1.6	18.6	6.98	4.47	18.6
88	GR11P2	East	G	11.7	-29.8	2.0	17.2	6.04	5.08	17.2
89	DT5P3	West	D	10.7	-30.5	1.6	14.1	4.18	3.97	9.1
90	DR5P3	West	D	13.6	-27.4	1.6	15.6	5.04	3.59	11.9
91	DT11P3	West	D	14.5	-30.5	1.5	17.1	5.97	4.64	12.6
92	DR11P3	West	D	13.3	-30.2	1.8	17.7	6.36	4.42	12.3
93	DT16P3	West	D	12.3	-27.0	2.2	18.1	6.63	5.71	12.4

94	DR16P3	West	D	12.2	-28.4	2.9	21.5	9.12	6.58	23.0
95	DT2OP3	West	D	12.1	-29.8	2.1	18	6.57	6.06	9.6
96	DR20P3	West	D	12.1	-29.7	2.4	20.4	8.28	6.75	17.7
97	NT5P3	West	N	14.5	-29.2	2.6	20.1	8.05	4.99	19.2
98	NR5P3	West	N	14.8	-33.6	2.3	20.4	8.28	5.67	7.1
99	NT11P3	West	N	14.0	-27.5	1.5	18.7	7.05	4.02	9.9
100	NR11P3	West	N	14.1	-30.7	1.6	19.3	7.47	6.08	12.9
101	NT16P3	West	N	13.3	-35.0	1.6	21.6	9.20	7.50	24.3
102	NR16P3	West	N	14.4	-29.8	1.8	19.6	7.69	5.14	15.5
103	NT20P3	West	N	13.0	-27.5	1.8	18.5	6.91	7.11	25.2
104	NR20P3	West	N	12.5	-30.2	3.0	19.8	7.83	6.09	15.4
105	TT5P3	West	T	12.8	-29.0	3.9	21.3	8.97	6.71	16.3
106	TR5P3	West	T	13.4	-30.0	1.7	17.9	6.50	6.47	13.7
107	TT11P3	West	T	13.9	-28.4	2.0	21.8	9.36	8.36	26.0
108	TR11P3	West	T	13.5	-28.6	1.7	24	11.20	8.19	18.6
109	TT16P3	West	T	14.7	-30.3	2.2	22	9.52	8.67	21.9

110	TR16P3	West	T	13.3	-29.6	2.6	24.5	11.60	8.72	10.2
111	TT20P3	West	T	12.7	-31.7	2.2	22.4	9.84	7.18	16.2
112	TR2OP3	West	T	13.4	-28.5	1.5	17.3	6.10	3.60	11.4
113	KT5P3	East	Kh	14.7	-31.6	2.5	16.5	5.59	3.51	13.8
114	KR5P3	East	Kh	12.7	-32.3	2.1	21.6	9.20	8.40	21.0
115	KT11P3	East	Kh	12.9	-27.4	2.1	17.7	6.36	4.98	14.3
116	KR11P3	East	Kh	12.9	-28.6	2.0	19.2	7.40	5.08	14.7
117	KT16P3	East	Kh	14.1	-25.7	1.6	15.2	4.80	3.05	9.8
118	KR16P3	East	Kh	14.7	-26.5	1.6	16.2	5.40	3.46	10.0
119	KT20P3	East	Kh	12.5	-28.0	2.1	15.1	4.74	3.95	11.3
120	KR20P3	East	Kh	14.6	-33.4	2.6	19.1	7.33	5.19	7.6
121	BT5P3	East	B	14.7	-27.7	1.8	15.5	4.98	3.76	12
122	BR5P3	East	B	12.5	-27.1	2.2	19.5	7.61	4.68	17.1
123	BT11P3	East	B	14.6	-27.7	1.5	20.4	8.28	3.51	12.9
124	BR11P3	East	B	15.0	-27.8	1.8	16.3	5.46	4.20	15.0
125	BT16P3	East	B	12.6	-27.1	2.0	17.6	6.30	5.02	13.2

126	BR16P3	East	B	13.4	-27.3	2.0	19.8	7.83	5.58	18.8
127	BT20P3	East	B	13.7	-29.0	1.6	20.3	8.20	5.95	15.2
128	BR20P3	East	B	14.7	-26.3	2.1	15.3	4.86	4.09	11.4
129	GT5P3	East	G	12.6	-28.6	2.2	18.4	6.84	4.72	13.9
130	GR5P3	East	G	13.7	-30.4	2.3	22.1	9.60	4.52	14.3
131	GT11P3	East	G	13.3	-30.2	1.9	19.0	7.26	4.34	13.1
132	GR11P3	East	G	13.4	-28.9	1.9	23.1	10.40	6.24	20.3
133	DT5P4	West	D	11.9	-28.2	1.5	19.1	7.33	4.56	10.8
134	DR5P4	West	D	11.3	-28.1	1.7	18.1	6.63	3.87	15.6
135	DT11P4	West	D	11.9	-29.7	2.0	18.2	6.70	4.98	13.2
136	DR11P4	West	D	11.1	-26.7	1.4	16.3	5.46	4.03	12.8
137	DT16P4	West	D	12.6	-31.4	1.7	19.4	7.54	6.42	9.8
138	DR16P4	West	D	12.4	-25.5	1.3	16.7	5.71	4.71	13.7
139	DT29P4	West	D	12.4	-29.9	1.7	17.0	5.91	6.08	10.1
140	DR20P4	West	D	11.9	-26.8	1.8	21.9	9.44	6.27	17.8
141	NT5P4	West	N	12.5	-28.6	2.0	20.3	8.20	6.17	17.2

142	NR5P4	West	N	13.2	-30.2	1.7	20.6	8.43	6.72	13.7
143	NT11P4	West	N	12.4	-29.1	1.6	19.1	7.33	5.71	11.3
144	NR11P4	West	N	13.3	-27.3	1.9	22.9	10.30	5.93	15.8
145	NT16P4	West	N	13.2	-32.2	1.9	22.5	9.93	5.52	11.1
146	NR16P4	West	N	13.6	-30.3	2.6	18.4	6.84	5.74	11.0
147	NT20P4	West	N	14.3	-28.3	2.0	18.7	7.05	5.09	11.6
148	NR20P4	West	N	13.3	-28.7	2.2	18.1	6.63	5.36	18.4
149	TT5P4	West	T	12.7	-27.1	1.8	19.8	7.83	6.83	19.9
150	TR5P4	West	T	14.1	-28.3	1.7	21.7	9.28	9.47	18.6
151	TT11P4	West	T	13.8	-30.3	2.1	21.3	8.97	7.41	24.2
152	TR11P4	West	T	13.6	-29.6	1.8	19.4	7.54	5.90	16.1
153	TT16P4	West	T	13.5	-27.4	2.4	22.7	10.10	8.79	25.2
154	TR16P4	West	T	11.9	-29.7	2.7	18.7	7.05	6.32	16.7
155	TT20P4	West	T	13.1	-32.8	2.6	21.9	9.44	6.63	19.5
156	TR20P4	West	T	12.9	-31.4	2.7	20.5	8.35	7.07	18.7
157	KT5P4	East	Kh	12.8	-27.0	1.8	15.7	5.10	4.03	11.4

158	KR5P4	East	Kh	13.2	-26.6	2.5	17.5	6.23	4.37	12.8
159	KT11P4	East	Kh	12.3	-27.0	2.2	17.9	6.50	5.33	11.7
160	KR11P4	East	Kh	12.9	-22.7	1.8	16.5	5.59	3.63	9.3
161	KT16P4	East	Kh	13.1	-28.5	2.3	17.6	6.30	4.76	14.9
162	KR16P4	East	Kh	11.2	-26.8	1.6	17.6	6.30	4.03	10.2
163	KT20P4	East	Kh	14.8	-33.7	1.9	17.4	6.17	5.07	5.3
164	KR20P4	East	Kh	13.2	-27.2	2.0	20	7.98	5.48	7.0
165	GT5P4	East	G	12.4	-30.0	2.1	19.9	7.91	4.58	9.7
166	GR5P4	East	G	13.3	-31.6	2.4	21.7	9.28	4.54	10.8
167	GT11P4	East	G	13.1	-28.7	2.1	20.4	8.28	4.33	13.0
168	GR11P4	East	G	13.0	-27.1	1.7	20.6	8.43	4.07	12.6

Chapter 5

5. Statistical analysis of results for fresh samples

The results obtained from different tests, given in the previous chapter, will be subjected to a one-way analysis of variance (ANOVA), correlation and regression analysis to determine the statistical significance on the quality of samples used in this study. Initially, the difference between the specific and representative samples will be examined followed by the difference between each plantation in both regions. Subsequently, the influence of different picks, age of trees and environmental factors on the physiochemical properties of *A. senegal gum* will be examined by comparing the two regions (i.e. west versus east).

The statistical analysis was performed at p value $\alpha = 5\%$ as described in section 3.2.7. The statistical analysis results will be presented and interpreted using the mean value together with the standard deviation which will usually be coded between two brackets.

5.1 Samples evaluation and comparison

5.1.1 Difference between representative and specific samples

As mentioned in section 3.1.2 two types of the samples were collected from six different plantations; namely specific (from one tree) and representative (form a group of trees within the same location). The main objectives here are to (i) obtain a sample for a given age set and (ii) to establish the extent of possible variations so that an accurate comparison between different age sets within the same location as well as from another location can be made. The result given in Table 4.1 were used to perform the statistical analysis by SPSS ANOVA-One way combined with descriptive analysis.

The protein content for both specific and representative samples gave an average value of 2.14 (0.41) % and 2.16 (0.46) % respectively. There is no statistically significant variation in the protein content between specific and representative samples ($F(1,166) = 0.264$, p

0.61). Additionally, both the specific and representative samples show similar trend for protein values as given in Figure 5.1.

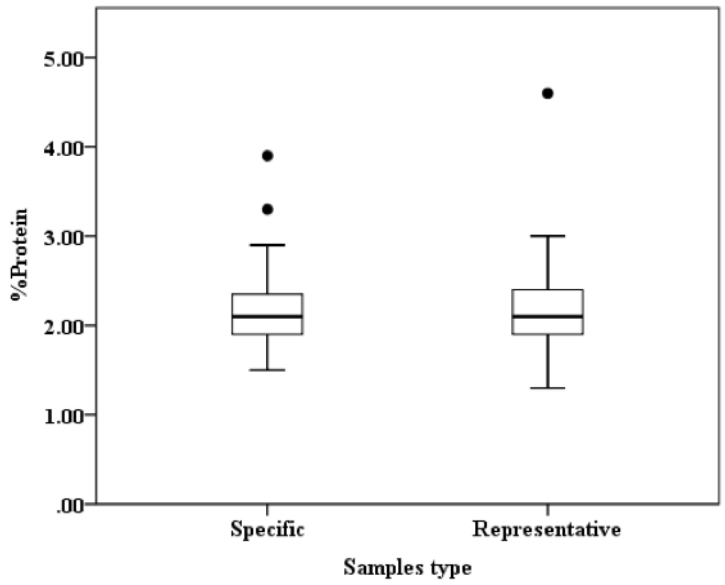


Figure - 5.1. A comparison of % protein (w/w) obtained from specific and representative samples. Where the dots (•) = outliers.

The same analysis was applied on the intrinsic viscosity results for specific and representative samples and the mean values of 19.49 (2.61) cm^3/g and 19.52 (2.18) cm^3/g were obtained. There is no significant statistical difference in the intrinsic viscosity value between specific and representative samples (F (1,166) = 0.012, p 0.91). The results are shown in Figure 5.2. According to the "Mark-Houwink" equation (see section 3.2.4.1) the value of the viscosity average molecular weight (M_v) is directly proportional with the intrinsic viscosity. The mean value for M_v for specific and representative samples was 7.56 x 10^5 (2.0 x 10^5) and 7.71 x 10^5 (1.60 x 10^5) g/mol respectively. (F (1,166) = 0.255, p 0.61) which indicates also no significant variation. Figure 5.3 shows a plot of intrinsic viscosity and M_v where the correlation between the two parameters for both set of samples are clearly evident.

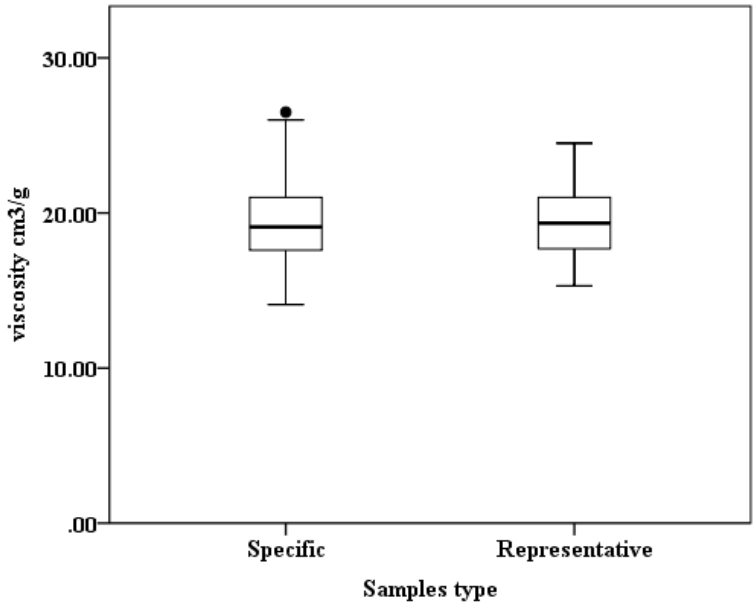


Figure - 5.2. Comparison of intrinsic viscosity results obtained from specific and representative samples.

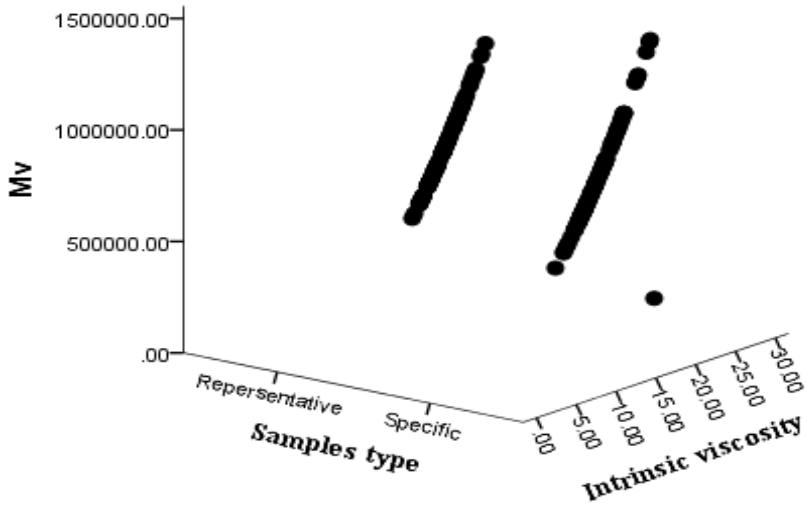


Figure - 5.3. Correlation between intrinsic viscosity and viscosity average molecular weight for specific and representative samples.

The results of the weight average molecular weight M_w obtained using GPC-MALLS technique described in section (3.2.5) were also analysed. A mean value of 7.57 x 10^5 (1.98

x 10^5) and 7.71 x 10^5 (1.59 x 10^5) g/mol for specific and representative samples respectively. There is no consequential distinction in M_w for the specific and representative samples (F (1,166) = 0.255, p 0.61). However, there is more coherent distribution in the representative samples compared to specific as shown in Figure 5.4.

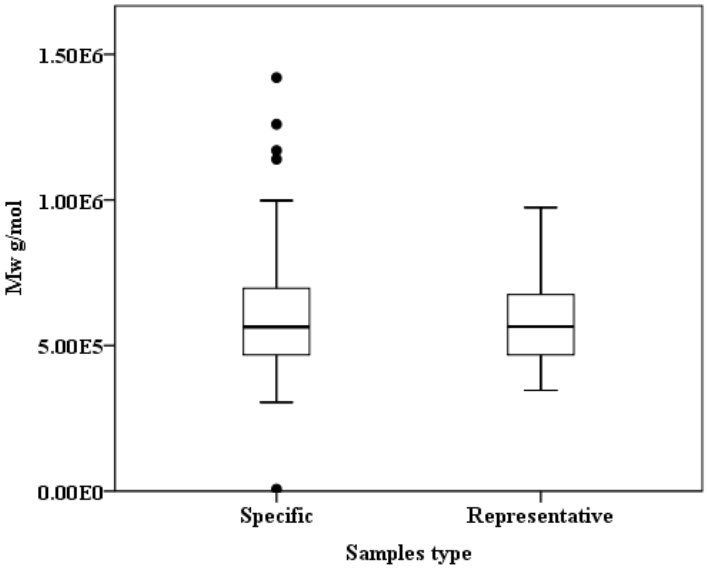


Figure - 5.4. Comparison of M_w results obtained from specific and representative samples.

Furthermore, the comparison between the proportion of the high molecular weight fraction (AGP) in both specific and representative samples showed that the average mass of the AGP were 15.4 (4.12) % and 14.8 (4.8) % respectively. The statistical analysis outcome revealed no significant differences between % AGP in specific and representative samples (F (1,166) = 0.430, p 0.51) and again there is more coherent distribution in the representative samples compared to specific as shown in Figure 5.5.

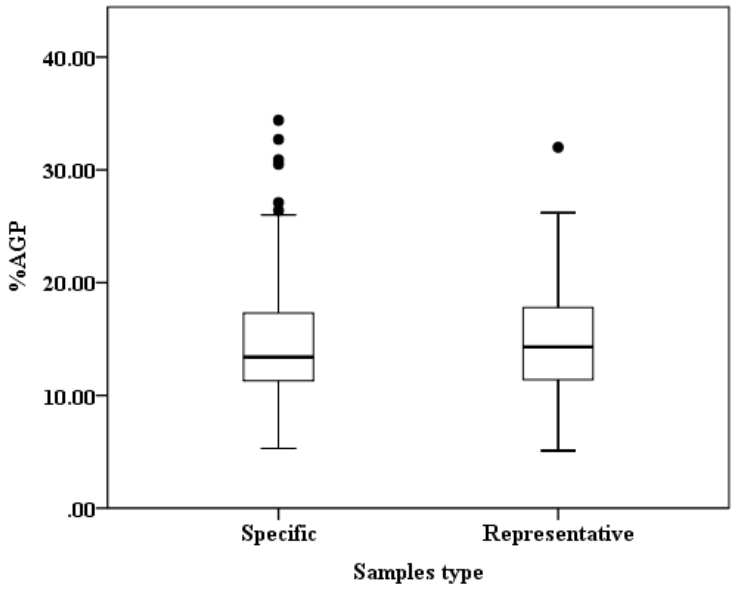


Figure - 5.5. Comparison of % AGP results between specific and representative samples.

5.1.2 Difference between samples from different locations

As already mentioned in (section 3.1.1), samples were collected from six different plantations in Sudan. The first three plantations were located in the western region and the last three in the eastern region. There are clear differences between the Western and Eastern regions in terms of environmental factors such as, soil type, average annual rainfall, average annual humidity and average annual temperature (see Table. 3-1).

One-way analysis of variance (ANOVA) is the statistical method used in this study to evaluate whether the variations between samples collected from different locations statistically significant or not.

In this section, the average value of all samples obtained from the same location is used in the statistical analysis. Protein content for samples obtained from different locations were found to be § for samples collected from AL-Demokeya, Nabag, Twobon, Khor-Donia, Bozy and Trees belt respectively. The highest value was obtained from Twobon plantations. Moreover, the plantations which allocated in western region showed higher values of % protein than plantations located in the eastern region. However, the values of protein from

different plantations were not statistically significant (F (5,162) = 1.51, p 0.189) as shown in Figure 5.6.

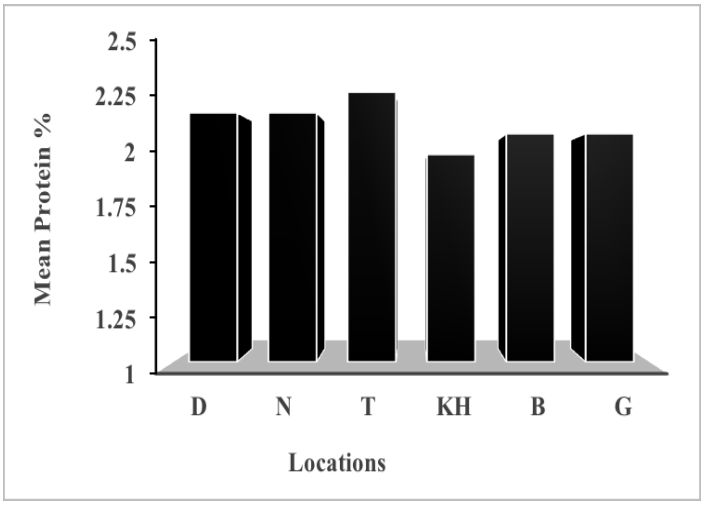


Figure - 5.6. % Protein for gum sample collected from different locations in Sudan.

The mean value of the intrinsic viscosity for samples acquired from different plantations were 19.8 (2.5), 20.1(1.4), 22.2 (2.3) ,18.0 (1.7), 18.1(1.6) and 19.7 (1.6) cm^3/g for samples collected from AL-Demokeya, Nabag, Twobon, Khor-Donia, Bozy and Trees belt respectively. The highest value of intrinsic viscosity was found to be in Nabag and Twobon plantations. There is a statistically significant difference in the intrinsic viscosity across the different plantations (F (5,162) = 19.395, p 0.000). Post – hoc Tukey HSD test showed that the plantations in the western region shown statistically significant difference in value of intrinsic viscosity than the plantations in the east region, however, there is no statistical variation in the intrinsic viscosity within the west or the east plantations as shown in Figure 5.7. Hence, the viscosity average molecular weight (M_v) value is directly correlated to the intrinsic viscosity, it is also showed significant variation between M_v values that obtained from different plantations (F (5,162) = 13.177, p 0.000) and showed the same trend of the intrinsic viscosity value with the location as shown in Figure 5.8.

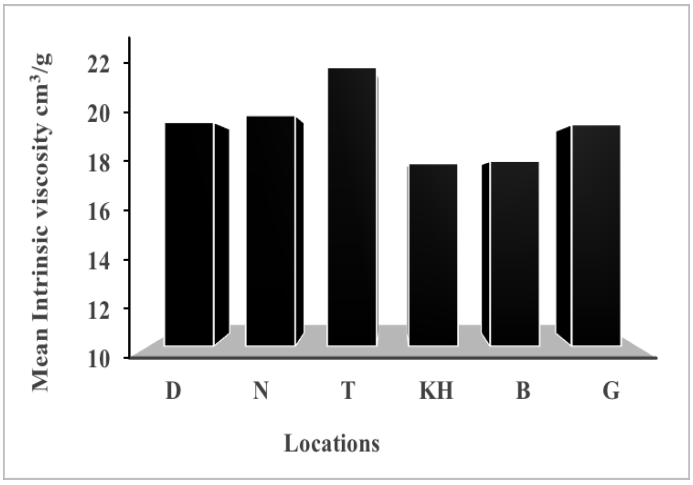


Figure - 5.7. Intrinsic viscosity values for gum sample collected from different locations in Sudan.

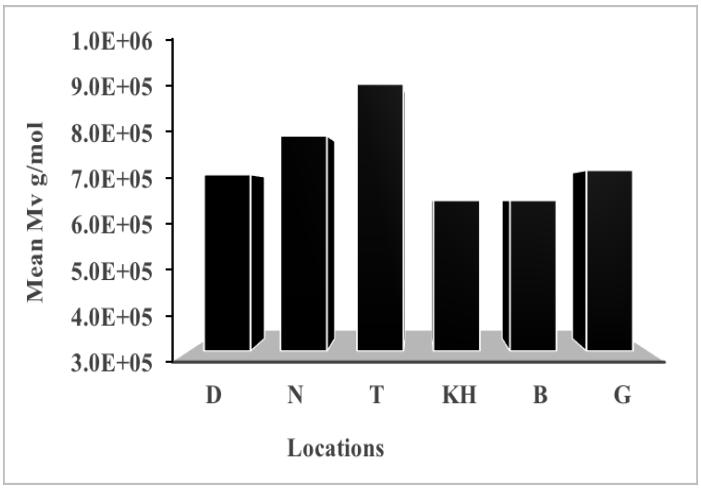


Figure - 5.8. M_v values for gum sample collected from different locations in Sudan.

The average value for weight average molecular weight (M_w) were 6.12 x 10^5 (2.1 x 10^5), 5.89 x 10^5 (2.52 x 10^5), 8.38 x 10^5 (1.95 x 10^5), 5.11 x 10^5 (1.23 x 10^5), 4.94 x 10^5 (0.79 x 10^5) and 4.83 x 10^5 (0.58 x 10^5) g/mol for samples collected from Al-Demokeya, Nabag, Twobon, Khor-Donia, Bozy and Trees belt respectively. There is a significant variation in M_w value across the different plantations (F (5,162) = 21.31, p 0.0007). The multiple

comparison test (Post – hoc Tukey HSD) revealed that samples obtained from the Western region were significantly higher than those from the Eastern region which is shown in Figure 5.9.

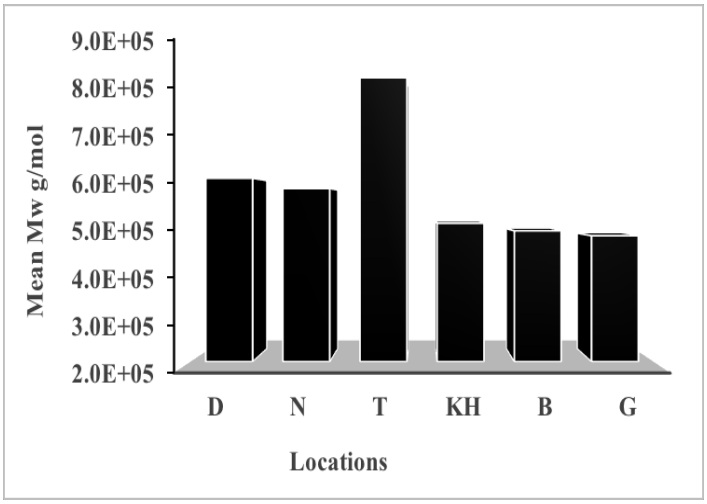


Figure - 5.9. M_w values for gum sample collected from different locations in Sudan.

The high molecular weight fraction (AGP) is one of the most important fraction of *Acacia* gum, was also examined. The average mass of the AGP were 15.2 (5.1) %, 15.2 (4.5) %, 20.3 (5.01) %, 11.6 (3.9) %, 13.2 (2.3) % and 14.1 (2.8) % for samples collected from Al-Demokeya, Nabag, Twobon, Khor-Donia, Bozy and Trees belt respectively. One-Way ANOVA combined Post-hoc Tukey HSD analysis showed that AGP mass result for samples collected from different location has similar trend to the results of M_w for the whole gum, where, samples collected from plantations located in the western region displayed statistically significant higher value of the AGP mass in comparison with samples collected from plantations in the eastern region ($F(5,162) = 12.246$, p 0.0000003). The results are shown in Figure. 5.10.

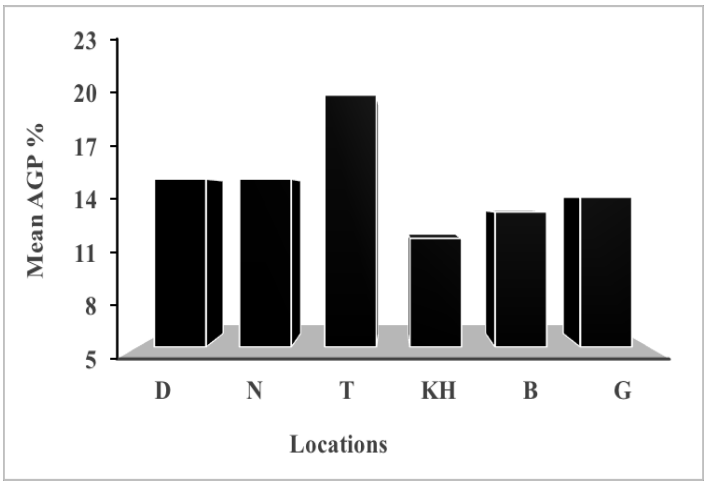


Figure - 5.10. % AGP values for gum sample collected from different locations in Sudan.

5.1.3 Conclusion

The evaluation and comparison of the samples from different type (specific and representative) and from different plantations using One-Way ANOVA combined with the Post-hoc Tukey HSD test indicated that:

(i) There is no statistically significant difference between the specific and representative samples through all the different parameters used in this study. Moreover, both the specific and representative samples showed similar trend to their correlation with the different locations. This finding, suggests that the sampling design model and processing which used in this study were very precise and performed carefully.

(ii) The variations between the results from different plantations across the all parameter used in this study are statistically very significant. However, the Post-hoc Tukey HSD showed that this variation is evident between the plantations in the east region and the plantations in the west region. There is no statistically significant variation within the respective plantation in the western and eastern regions. These results are in good agreement with previous results reported in section 1.8.

Therefore, based on the above two findings, the following section will deal with the result of each region as one unite. Thus, the impact of the independent variables such as the environmental factors, different picks, different age of trees and storage conditions on the physiochemical and functional properties throughout chapters 5, 6 and 7 will be examined and compared on the basis of the average (mean) value obtained from all samples in the eastern and western regions.

5.2 The influences of different picks, age of trees and environmental factors on the physiochemical properties of *A. senegal* gum

5.2.1 Difference between various picks within one region and other regions

The picking of exudates gum from *Acacia* trees, following tapping, is a consecutive process whereby the number of picks vary from place to another. This process mainly depends on the variation of environmental factors such as temperature, humidity and rainfall (Imeson, 1992). Typically, the intervals between picks (collection) is about two weeks but it could vary and in some places significantly. In some areas, it may go up to six interval picks (see section 2.6.1). According to the local knowledge a change in the temperature during the picking season has been noted to affect the quality and quantity of the subsequent pick. Therefore, this aspect has been included in this study to determine the statistical difference between samples collected during the same season. Furthermore, comparison between the two regions (i.e. East and West of Sudan) was also included in this study (see section 2.3.1). The mean values of the protein content in the western region for gum samples collected from four different picks were 2.5 (0.3) %, 2.3 (0.6) %, 2.1 (0.6) % and 2.0 (0.4) % for 1st, 2nd, 3rd and 4th pick respectively. The results of protein content demonstrated gradual decrease in the value as a function of the picking interval. There is a significant variation between the protein values obtained from various picks ($F(3,92) = 6.364$, p 0.001). Post – hoc Tukey test showed that the variation is significant in particular between 1st and 2nd picks compared to the 3rd and 4th picks. The same analysis was performed on samples collected from the Eastern region and values of 2.1 (0.3) %, 2.1 (0.3) %, 2.0 (0.3) %, and 2.0 (0.3) % for 1st, 2nd, 3rd and 4th pick respectively were obtained. Statistically there is no significant variation between these values ($F(3,68) = 3$, p 0.07). Nevertheless, the value of the protein content in both regions displayed the same trend where it is high in the first pick and then steady decreased thereafter. The results demonstrate clearly that the protein content for the

samples in the Western region is slightly higher than those from the Eastern region as shown in Figure 5.11.

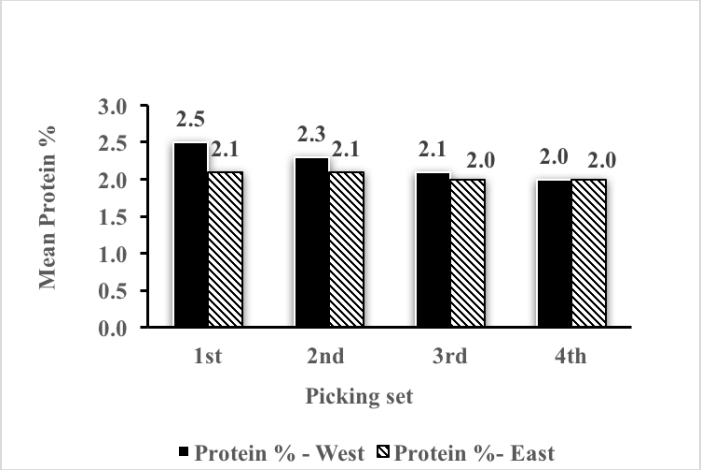


Figure - 5.11. Picking sets versus % protein in gum arabic samples collected from the Western and Eastern regions in Sudan

The intrinsic viscosity mean values in the western region for samples collected from the various picks were 20.7 (2.4), 21.3 (2.8), 19.7 (2.5) and 19.7 (1.9) cm^3/g for 1^{st}, 2^{nd}, 3^{rd} and 4^{th} pick respectively. Statistically there is consequential difference between the various picks in western region (F (3,92) = 3.487, p 0.051). Multiple comparison Post-hoc Tukey test confirmed that the significant difference in samples from the two first picks (highly viscous) compared with those from the third and fourth picks. This difference in viscosity between the different picks can be possibly attributed to the higher protein already noted above. In the Eastern region, the mean values of intrinsic viscosity were 18.7 (1.3), 18.6 (1.3), 18.4 (2.4) and 18.0 (1.9) cm^3/g for 1^{st}, 2^{nd}, 3^{rd} and 4^{th} pick respectively. The results also showed gradual decrease as function of the picking interval as the same as noted in the western region. However, there is no statistically difference in viscosity value across the different picks (F (3,68) = 0.548, p 0.7). In both regions, the viscosity values of the 1^{st} and 2^{nd} picks are higher than the 3^{rd} and 4^{th} picks. Moreover, the viscosity values in the western

region were also higher than those in the Eastern region throughout the all picks as shown in Figure 5.12. The significance of these results is the confirmation of an established local knowledge about the higher viscosity typically associated with the Western region compared to the Eastern region in Sudan.

The intrinsic viscosity value is used in the Mark-Houwink equation (see section 3.2.4.1) to calculate the viscosity average molecular weight to further examine the difference between the various picks from the two regions. The mean values of M_v in the western region were found to be 8.08 x 10^5 (2.1 x 10^5), 9.06 x 10^5 (2.2 x 10^5), 7.82 x 10^5 (1.8 x 10^5) and 7.83 x 10^5 (1.4 x 10^5) g/mol for the 1st, 2nd, 3rd and 4th pick respectively. In the Eastern region, the value of M_v were 7.08 x 10^5 (0.92 x 10^5), 6.61 x 10^5 (0.91 x 10^5), 6.2 x 10^5 (1.7 x 10^5) and 7.0 x 10^5 (1.3 x 10^5) g/mol for the 1st, 2nd, 3rd and 4th respectively. In the Western region, there is a slightly significant variation in the viscosity average molecular weight ($F(3,92) = 2.968$, p 0.07), however, in the Eastern region there is no significant variation in the viscosity average molecular weight values ($F(3,68) = 0.55$, p 0.6) as shown in Figure 5.13.

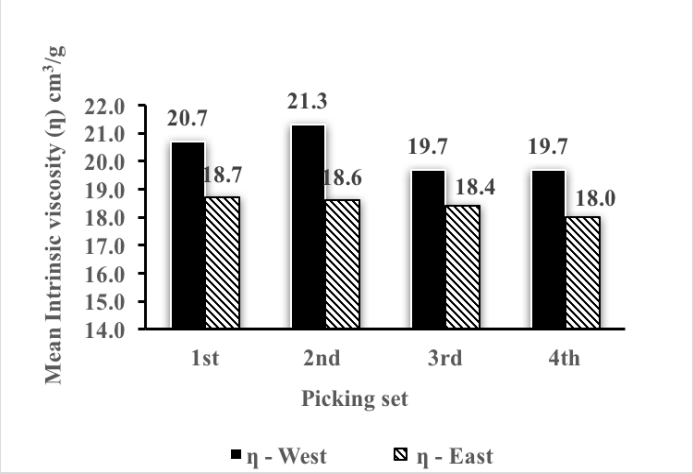


Figure - 5.12. Picking set versus intrinsic viscosity in the Western and Eastern regions in Sudan.

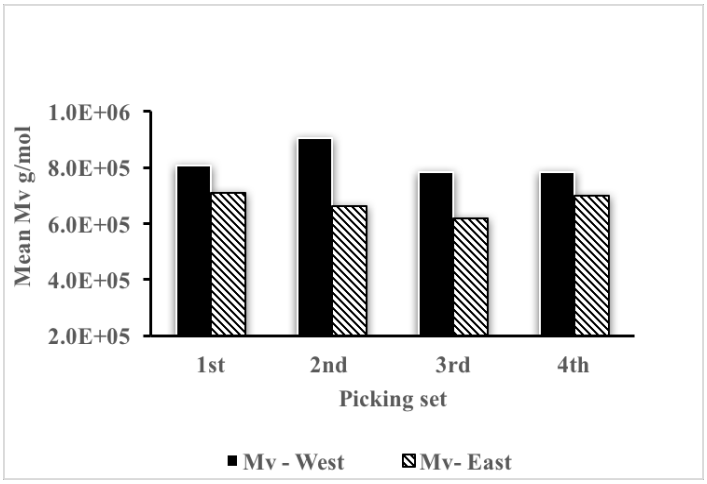


Figure - 5.13. Picking set versus M_v for samples collected from the West and East regions in Sudan.

A more accurate (absolute) measure of the molecular weight is the weight average molecular weight (M_w) obtained by GPC-MALLS (see section 3.2.5). Here, this parameter was used to conduct the same analysis as given above. The mean value of M_w from the western region for samples obtained from various picks were 8.10 x 10^5 (2.1 x 10^5), 7.25 x 10^5 (2.4 x 10^5), 5.80 x 10^5 (2.0 x 10^5) and 6.07 x 10^5 (1.3 x 10^5) g/mol for 1st, 2nd, 3rd and 4th pick respectively. Those values displayed significant variation with the picking interval ($F(3,92) = 6.955$, p 0.0003). Using Post-hoc Tukey test indicated precisely the variation is very clear between 1st pick and 3rd pick and 1st pick with 4th pick. In the eastern region, the mean values of M_w were 5.59 x 10^5 (0.80 x 10^5), 4.97 x 10^5 (0.81 x 10^5), 4.71 x 10^5 (1.2 x 10^5) and 4.52 x 10^5 (0.56 x 10^5) g/mol for the 1st, 2nd, 3rd and 4th pick and also showed significant variation as function of the picking interval ($F(3,68) = 4.561$, p 0.006). Here also, using Post-hoc Tukey test showed there was a clear statistical variation between 1st pick and 3rd pick and, 1st pick and 4th pick. Remarkably, the same trend of M_w correlation with the various picks obtained in both regions despite the fact that M_w values from various picks in the west region are considerably higher than those from the eastern region the

highest value is in the first pick and then decrease thereafter up to the fourth pick as shown in Figure 5.14.

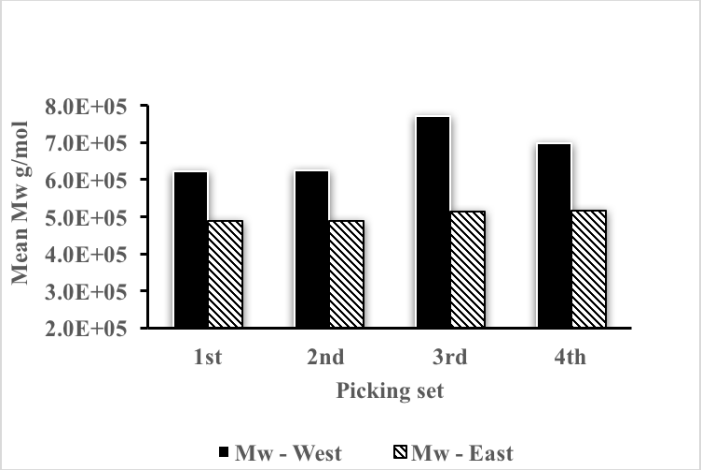


Figure - 5.14. Picking set versus M_w for gum Arabic samples collected from the Western and Eastern regions in Sudan.

The means mass value of the high molecular weight fraction, arabinogalactan-protein fraction (% AGP) in the Western region for samples acquired from various picks were 18.4 (4.9) %, 18.1 (6.0) %, 15.5 (4.4) % and 15.5 (4.2) % for the 1st, 2nd, 3rd and 4th pick respectively. These values of %AGP do not demonstrate any consequential statistical difference (F (3,92) = 1.826, p 0.15). On the other hand, the mean values of 13.9 (3.4) %, 13.0 (4.3) %, 12.2 (2.8) % and 10.7 (2.7) % for the 1st, 2nd, 3rd and 4th pick respectively were obtained from the eastern region with (F (3,68) = 1.976, p 0.13) indicating no statistically significant variation also. Nevertheless, the values of %AGP in both regions displayed the same trend where %AGP in the first pick is high and then decreased thereafter. Additionally, the values of %AGP in West region are substantially higher than the value of %AGP in the East region. The results are shown in Figure 5.15.

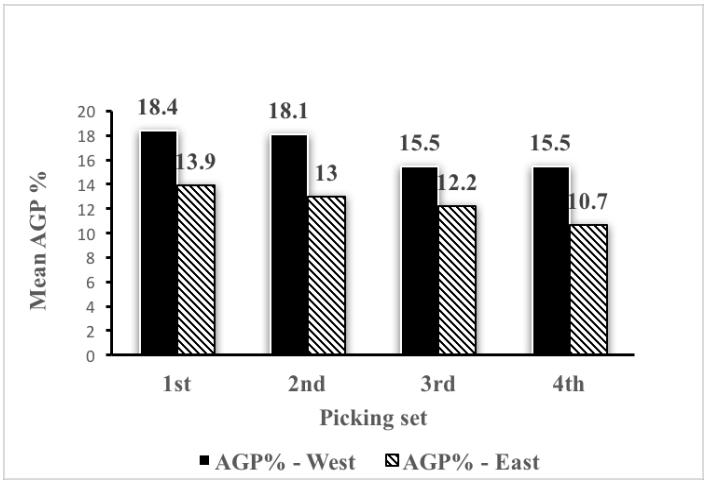


Figure - 5.15. Picking set versus the mean % AGP in the Western and Eastern regions in Sudan.

5.2.2 Difference between tree age set within one region and another region

As described previously (see section 2.6.1) *Acacia* trees start to exudate gum at an age of ~ 5 years and continues to over 25 years. In this study, samples were collected from 4 different sets of ages of trees, from both Western and Eastern regions in Sudan. The objective is to examine the possible influence of tree age on the physiochemical and functional properties of Gum arabic samples used in this study. The main parameters (i. e. protein content, intrinsic viscosity, molecular weight and % AGP fraction) will be used here similar to the statistical method employed in the previous section. The mean values of % protein for samples obtained from the western region and collected from different trees age were 2.2 (0.6) %, 2.1(0.7) %, 2.2 (0.4) % and 2.3 (0.4) % for trees age 5, 10, 15 and 20 years respectively, those values of protein content in the western region showed very close similarity and there is no significant variation found as function of trees age ($F(3,80) = 0.735$, p value= 0.53). In the eastern region, the mean values were 2.1 (0.2) %, 2.0 (0.3) %, 2.0 (0.3) % and 2.1 (0.2) % for trees age 5, 10, 15 and 20 years respectively. The result in the eastern region also showed close similarities and there is no significant variation (F

(3,61) = 1.663, p value 0.2). However, in the both regions the highest values of % protein were found to be in sample collected from the oldest trees 20 years old as shown in Figure 5.16.

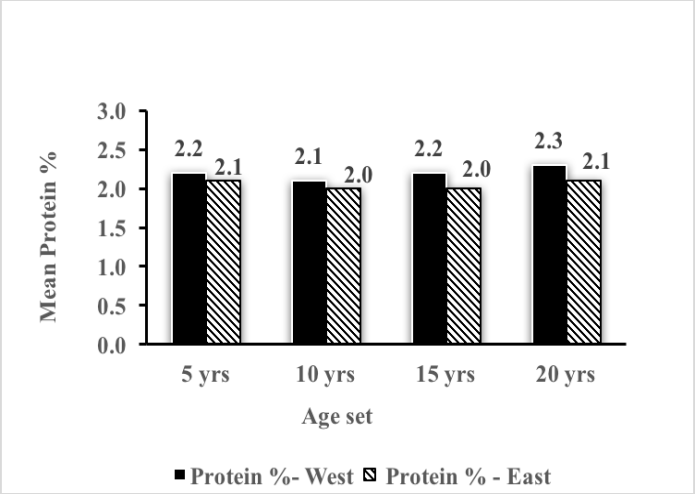


Figure - 5.16. Tree age set versus % protein for gum arabic samples collected from the Western and Eastern regions of Sudan.

The Intrinsic viscosity means values 19.5 (2.6), 19.8 (2.4), 21.3 (2.3) and 20.8 (1.8) cm^3/g for trees age 5, 10, 15 and 20 years respectively. As it can be seen, the gum produced from the youngest trees 5years old and 10 years old were less viscous than those from older trees 15 years old and 20 years old. The results revealed statistically significant variation in intrinsic viscosity as function of different age of trees (F (3,8) = 2.717, p 0.05). Post – hoc Tukey test showed that viscosity of 15 years old tree is significantly higher than the viscosity obtained from 5 years' tree. In the eastern region, the mean values were 18.6 (1.8), 18.6 (1.7), 18.2 (1.9) and 17.5 (1.6) cm^3/g for trees age 5, 10, 15 and 20 years respectively. These values showed no statistical meaningful difference (F (3,61) = 0.87, p 0.5). Noticeably, the values of the intrinsic viscosity in each region displayed distinctive features whereas a clear gradual increase in the western region up to 15 years followed by slight reduction. On the other hand, in the Eastern region the values were almost comparable.

However, the intrinsic viscosity values are higher in the western region than the eastern region as shown in Figure 5.17. Despite the correlation between the intrinsic viscosity and viscosity average molecular weight (M_v), the M_v values in both regions showed no statistical considerable variation (p value = 0.4) and (p value = 0.5) for Western and Eastern region respectively. Moreover, the values of M_v in both regions do not show the same trend as shown in Figure 5.18.

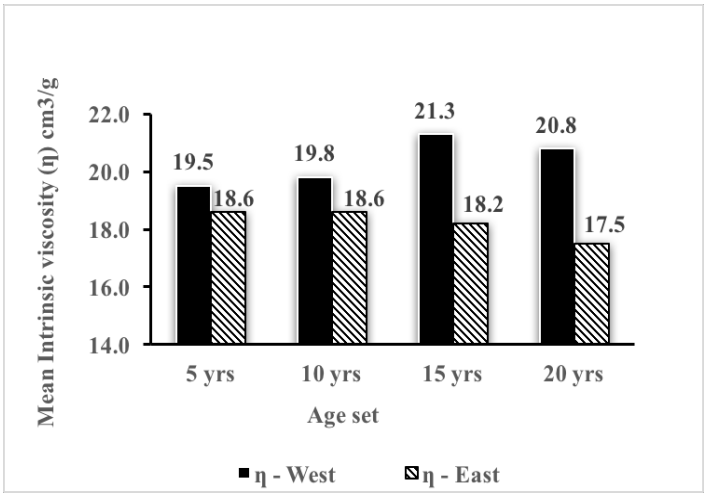


Figure - 5.17. Tree age set versus intrinsic viscosity for gum arabic samples collected from the western and eastern regions of Sudan.

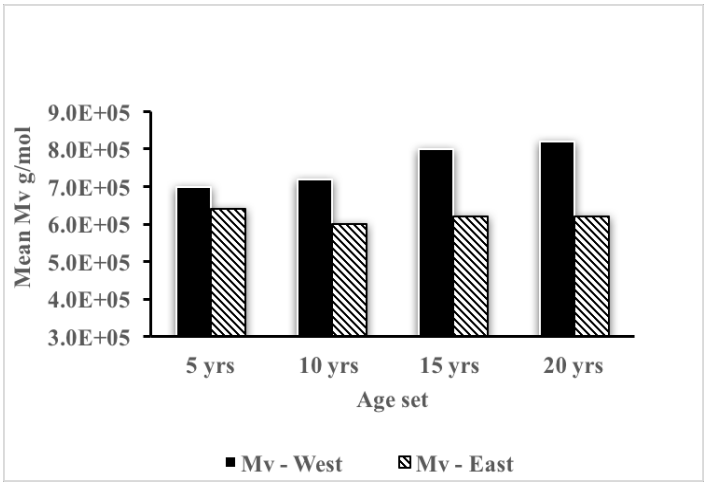


Figure - 5.18. Tree age set versus M_v for gum arabic samples collected from western and eastern regions in Sudan.

The mean values of (M_w) for samples obtained from western region and collected from *Acacia* trees of different ages, were 6.23 x 10^5 (1.6 x 10^5), 6.26 x 10^5 (1.9 x 10^5) 7.72 x 10^5 (2.3 x 10^5) and 6.98 x 10^5 (1.8 x 10^5) g/mol for trees age 5, 10, 15 and 20 years respectively. Trees aged 10 and 15 years old showed the highest value of M_w than 5 and 20 years old. There is statistically significant difference in M_w values as function of different trees age in the western region (F (3,80) = 3.091, p 0.03). Multiple comparisons test (Post-hoc Tukey) revealed that the M_w value of 15 years is significantly higher the M_w values of 5 and 10 years old. The same analysis was performed on samples from the Eastern region. Here, the M_w values were 4.88 x 10^5 (1.0 x 10^5), 4.88 x 10^5 (0.94 x 10^5), 5.15 x 10^5 (1.2 x 10^5) and 5.17 x 10^5 (0.7 x 10^5) g/mol, for trees age 5, 10, 15 and 20 years respectively. The result revealed the same trend identified in the western region where the highest values of M_w were associated with samples obtained from trees age 10 and 15-year-old. Nevertheless, the result showed no statistical significant variation in M_w as function of different age of trees. Generally, the M_w value in western region is significantly high than those in the eastern region through all the different age sets. The correlation between different age of tree from west and east regions in Sudan and M_w values is shown in Figure 5.19.

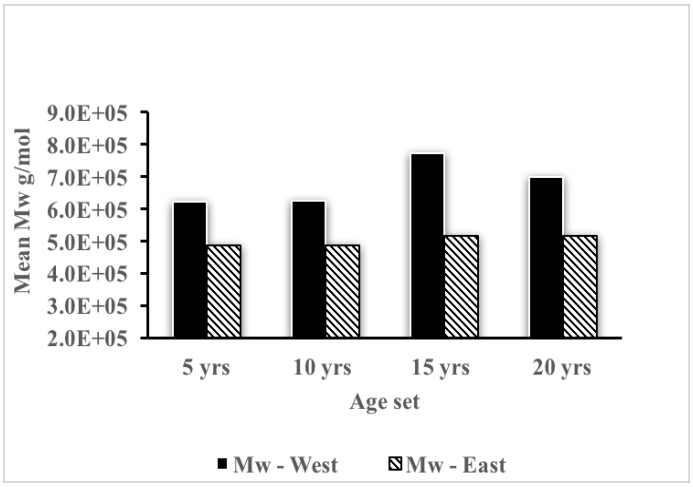


Figure - 5.19. Correlation of tree age set versus M_w in Western and Eastern regions in Sudan.

The mean mass values of the high molecular weight fraction (AGP) obtained from the western region were 15.2 (4.4) %, 15.5 (5.1) %, 19.5 (4.6) % and 18.8 (3.5) % for samples from trees age 5, 10, 15 and 20 years respectively. The highest values of % AGP were found to be in the oldest trees (i.e. 15 and 20 years old). The values of % AGP which collected from different age of trees in the western region revealed significant statistical variation (F (3,80) = 2.391, p 0.04). While in the eastern region the mean values of % AGP were 12.3 (2.9) %, 12.7 (3.0) %, 12.9 (2.9) % and 12.7 (3.4) % for samples from trees age of 5, 10, 15 and 20 years respectively. The % AGP values in the eastern region showed the same where the highest value of % AGP was from the oldest trees 15 years old. The result of % AGP acquired from different age of trees in the eastern region exhibited no significant statistical variation (F (3,61) = 1.728, p 0.171). Generally, the %AGP values in the western region significantly higher than those in the eastern region through all age sets. The correlation between the % AGP and the age of the trees in both regions is shown in Figure 5.20.

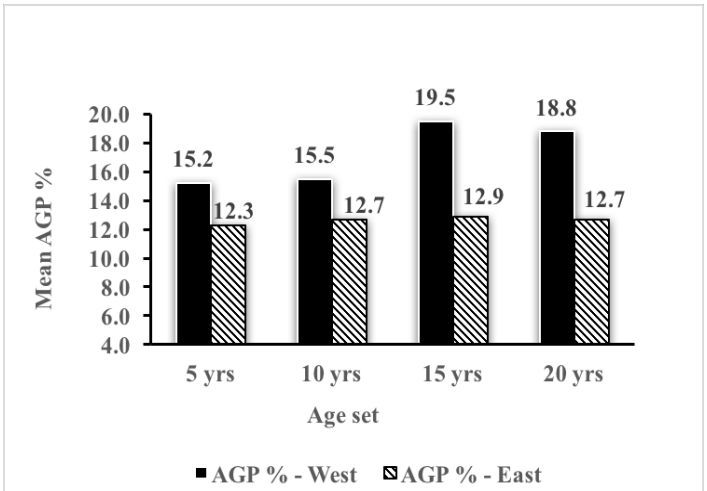


Figure - 5.20. Tree age set versus % AGP for Gum arabic samples collected from western and eastern regions in Sudan.

5.2.3 The influences of the environmental factors on the physiochemical properties

As mentioned in section 2.3.1 the western and eastern regions in Sudan differs in so many environmental factors such as soil type, annual average rainfall, annual average temperature and annual average humidity. The previous sections (5.3 and 5.4) gave strong evidences of the differences in the physiochemical properties between the gum collected from different picks and different age of trees in western and eastern regions. However, in this section the influences of the different environmental factors such as soil type, rainfall, temperatures and humidity on the physiochemical properties will be examined using correlation test and linear regression analysis. To identify the correlation between these factors and the different physiochemical properties and to suggest the possible significant model equation and predictors. All the categorical variables were coded using a dummy variable coding method.

5.2.3.1 The influences of the environmental factors on the protein content

Pearson correlation factor (r) showed positive correlation for % protein with soil type r = 0.18. It also showed negative correlation for % protein with rainfall, temperature and humidity r = -0.21, - 0.06 and -0.22 and respectively. However, only the correlation between % protein and soil type and rainfall is statistically significant p = 0.011 and 0.051 respectively. Moreover, the regression analysis revealed the significant regression equation was (F (4,163) = 2.503, p 0.044) with an r^2 (0.14). The regression model for predication the % protein is statistically significant and 14% of variation in the % protein can be explained by environmental factors. Participants predicated % protein as in following model equation:

% protein = 0.865 + 0.140 s + 0.03 t + 0.023h – 0.001r [5-1]

Where s is the soil type and according to a dummy variable coding s = 1 for sand soil and s= 0 for the clay soil, t = temperature degrees, h = humidity in % and r = rainfall in mm. This regression model equation (5-1) indicates that the protein content for gum collected from sandy soil relatively higher by 0.14 % than the clay soil, 1degree increase of temperature relatively increases the protein content 0.03%, 1% increase in humidity relatively increases the protein content 0.023 % and 1mm increase in rainfall relatively decreases the protein content by 0.001%.

5.2.3.2 The influences of the environmental factors on the intrinsic viscosity

Intrinsic viscosity values vary widely from area to another and from season to season due to the influences of the environmental factors. The impact of this factors on viscosity value was examined using correlation and regression analysis for Gum arabic samples collected from different locations. Pearson correlation factor (r) revealed that there is positive correlation between the viscosity value, soil type and temperature r = 0.499 and 0.23 respectively. Also, there is negative correlation between the viscosity value, rainfall and

humidity r = - 0.11 and – 0.06 respectively. However, only the correlation of viscosity with the soil type and rainfall is statistically significant p = (0.001and 0.03) respectively.

Moreover, a significant regression equation was found (F (4,163) = 8.712, p 0.000) with an $r^2 = 0.279$. The regression model for predicting the viscosity value is statistically significant and indicated that 27.9% of the variation in the viscosity value can be explain by the soil type, temperature, rainfall and humidity. These factors predicated the intrinsic viscosity value as in the following equation:

Intrinsic viscosity (cm^3/g) = 11.49 + 1.96 s + 0.151 t + 0.10 h – 0.003 r [5-2]

The model equation (5-2) suggested that the intrinsic viscosity of *Acacia gum* collected from the sand soil is relatively higher by 1.96 cm3/g than gum collected from the clay soil, 1 degree increase in temperature relatively increases the viscosity 0.151 cm^3/g, increase of 1% in humidity relatively increases the viscosity 0.01 cm^3/g and 1mm increase of rainfall relatively decreases the viscosity by 0.003.

5.2.3.3 The influences of the environmental factors in molecular weight and molecular weight distribution.

The weight average molecular weight (M_w) and molecular weight distribution (% AGP) of *Acacia* gum vary widely through the gum belt in Sudan. It has been reported that M_w and %AGP can possibly be affected by temperature and humidity before or after the picking of the gum nodules. This effect may lead to change in the value of M_w and %AGP due to redistribution of the molecules. Therefore, the impact of the environmental factors on M_w and % AGP was examined using correlation and regression analysis for samples obtained from different locations. Pearson correlation factor (r) indicated positive correlation for M_w value with soil type and humidity r = 0.588 and 0.126 respectively, and negative correlation for M_w value with temperature and rainfall r = 0.188 and 0.197. Nevertheless, only the correlation of M_w value with the soil type and the rainfall is

statistically significant. Furthermore, the regression analysis revealed a significant equation (F (4,163) = 13.705, p 0.0001) with an r^2 = 0.352 which indicate that 35.2% of the variation in the M_w value can be explained by the environmental factors and they can possibly be predicated as given in the following equation:

M_w g/mol = - 25735.6 + 245623.2 s + 8709. 8 t + 12179.8 h – 400.04 r [5-3]

The above regression model equation (5-3) suggested that the M_w value of *Acacia* gum collected from the sand soil is relatively higher by 2.456 x 10^5 g/mol than gum collected from the clay soil, 1 degree increase in temperature relatively increase the M_w 8709.8 g/mol, increase of 1% in humidity relatively increase the M_w 12179.8 g/mol and 1mm increase of rainfall relatively decrease the M_w by 400.04 g/mol.

The molecular weight distribution (%AGP) showed the same correlations of M_w with the environmental factors. A significant regression equation (F (4,163) = 7.307, p 0.0003) with an r^2 = 0.252 which means that 25.2% of variation in the %AGP can be explained by the environmental factors. They can possibly be predicated in the following regression model equation:

%AGP = 10.18 + 3.2 s +0.06 h + 0.079 t – 0.001r [5-4]

The regression equation model (5-4) suggested that %AGP of *Acacia* gum collected from the sand soil is relatively higher by 3.2% than gum collected from the clay soil, 1 degree increase in temperature relatively increases %AGP 0.079 %, increase of 1% in humidity relatively increase the %AGP 0.06 % and 1mm increase of rainfall relatively decrease the %AGP by 0.001%.

5.2.3.4 Conclusion

The physicochemical properties for the exudate gum are significantly affected by the picking interval. This impact was clearly demonstrated in the protein content, viscosity and molecular weight, and molecular weight distribution. In both regions, the first pick gave highest value of % protein, viscosity, M_w and % AGP and then decreased thereafter in the fourth pick. This phenomenon could be explained due to that fact any plants are able to exude proteases enzyme from their roots and use them to create a pool of accessible nitrogen nutrition (Godlewski & Adamczyk, 2007), and thereafter take up this nitrogen nutrition, but with the significant differences in effectiveness (Weigelt, King, Bol et al., 2003). Many factors affect the efficiency of the nutrition uptake , such as the competition with microorganisms which increases when the tree is injured (tapping to promote gummosis) and decrease of the amino acid concentration in soil due to the negative proportion between the exude of the proteases enzyme from the roots and the exudation of the gum (Lipson & Nasholm, 2001). This conclusion, consolidated the theory that the formation of gum is a result of microbial infection of the injured tree, and the plant synthesise the gum exudates in order to seal off the infected section and prevent further invasion of the tissue (see section 2.1).

Acacia gum exudates from trees that vary in their age. The analysis of the results shown that the physiochemical properties of *A. senegal* gum exhibit some variation depending on the age of the tree. There are significant differences in properties of gum from western region, where, 15 years old tree produced gum which significantly high in viscosity and molecular weight than the youngest tree of 5 years old and oldest tree of 20 years old. However, in the eastern region the result disclosed some variation but not statistically significant. Nonetheless, the results in both regions displayed similar trend, whereas, the physiochemical values increased from youngest trees to reach the peak at certain age and

decreased thereafter. This finding is in a good agreement with previous reports (Anderson et al., 1968; Idris et al., 1998). There is no clear scientific explanation for this finding so far. However, further work in *Acacia* tree morphology and gummosis is required before any firm conclusions can be drawn.

Analysis of the results for samples collected from the two regions demonstrated that the physiochemical properties present significant variation depending on the location of the tree. Gum from western region revealed significantly high values of different properties than gum from the eastern region.

The influences of the environmental factors on the physiochemical properties examined using regression analysis and indicated negative correlation between protein content with rainfall, humidity and temperature factors. Positive correlation between viscosity, molecular weight and AGP mass with soil type, humdity and temperature factors, and negative correlation between viscosity, molecular weight and AGP mass with rainfall factor. However, the most influential environmental factor caused this variation in the physiochemical properties is the soil type, hence the gum produced from the sandy soil in the western region showed significantly high values of protein, viscosity, molecular weight and AGP mass than gum produced from clay soil in the east region. This can be explained due to the fact that *A*. *senegal* gum yield process and nutrition seems to be highly affected by the soil water content at different soil depth. High permeability of sand soil because of the big size of particles relatively increase the availability of water during the drought period at reachable depth. However, the clay soil has very limited permeability and most of water evaporate from the top surface of clay soil. Additionally, cracks in clay soil appear shortly after the rain season and that leads to evaporation of the part of water in the soil depth.

The regression analysis suggested model equations to predict the physiochemical properties values depend on the environmental factors. Some of these equations can explain a very

high percentage in the variation of properties. Again, the result of the regression analysis confirmed that the soil is the most significant predicting factor followed by rainfall and temperature.

Chapter 6

6. Effect of storage

Naturally, as the *Acacia* tree grows the molecular weight of the exudate gums change proportionally due to joined of small sub-units into large units (Al-Assaf et al., 2007). The change in composition of produced gum continues after harvesting as well (Phillip, 1993). The protein content, optical rotation and intrinsic viscosity change significantly during storage of the gum, symptomatic to a continuing joined of small sub-units into large units which known as molecular aggregation process (Al-assaf., et al 2007). Storage of the gum at cold environment (4 ℃) for a period of 20 months showed no change at all. Contrariwise, storage of gum at constant temperature humidity caused significant change in gum composition (Al-assaf., et al 2012). Therefore, gum composition can possibly change naturally on maturing if subjected to certain environmental factors such as temperature and humidity. In addition to the biological factors that associated with the growth of the trees. The change achieved also, in *A*. *senegal* in particular, by increasing aggregation of the fraction AG which associated with small amount of protein to form further amounts of AGP fraction. Al-Assaf et al, mimicked those conditions which occurred naturally during the maturation process by using purely physical and environmental treatment which does not involve chemical agent and no new chemical groups are introduced or removed and it is achieved by a redistribution of existing components through subjected the gum samples to strictly controlled conditions of temperature and humidity (Al-assaf et al., 2003; Hayashi, 2002) as shown in Figure 6.1.

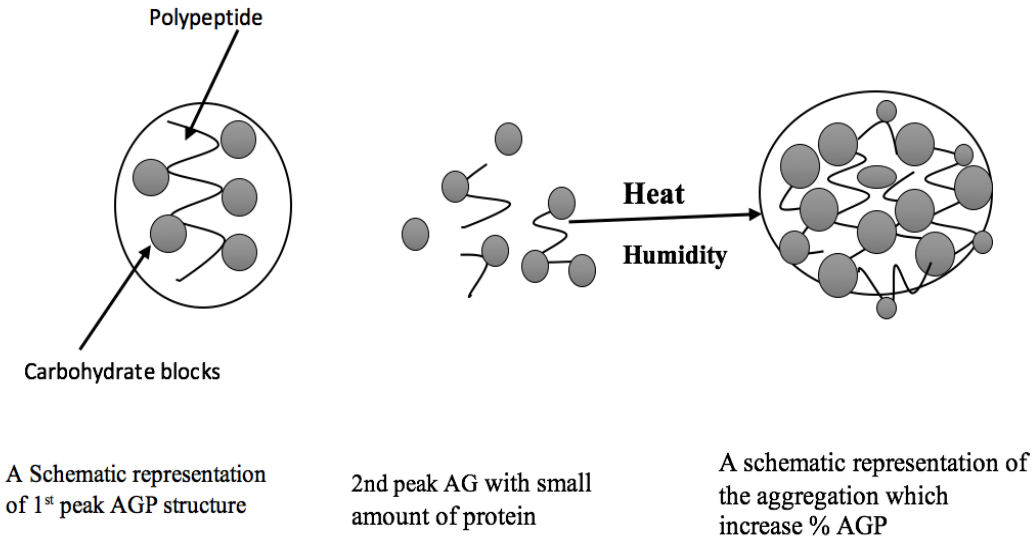


Figure - 6.1. Aschematic illusitrated the maturation process in *A. senegal* gum.

In this study, the impact of the storage condition on the properties of *A. senegal* gum in particular the molecular weight and molecular weight distribution was investigated. The effects of storage condition examined by the analysis of dry solid samples of *A. senegal* gum which subjected to natural maturation process by storage in different locations for period of five years (see section 3.1.4). Only the result of limited number of stored samples (44 samples) were used to evaluate the impact of the storage condition on the molecular weight and molecular weight distribution and compared with initial results of the fresh sample which listed in Table (4-1). Stored samples were selected based on the molecular weight value level to cover the high molecular weight, medium molecular weight and low molecular weight samples, with consideration to other factors such as different regions, age set and picking interval. Samples stored in different locations and kept in cotton bags in form of mixed lumps and nodules with small broken gum imitative the famous commercial grate cleaned gum, after five years' samples were kibbled to less than 1mm size just before

they used to carry the test. However, samples stored in UK were examined also after three years and that due the availability of this samples in the laboratory.

GPC method used to evaluate the different of molecular weight and molecular distribution. One-way ANOVA statistical analysis used to evaluate the nature of the differences between the mean values of the molecular weight (M_w) and the mean mass value of the high molecular weight fraction (AGP) of *A. senegal* gum for the fresh and stored samples. The descriptive statistic associating with the mean value of M_w and % AGP across the fresh and stored samples shown in Table 6-1 and 6-2.

Table. 6-1 Comparison between the mean M_w value for fresh samples and stored.

Sample details	M_w g/mol	SD
Fresh	5.96×10^5	1.41×10^5
Stored in UK 3 yrs.	6.54×10^5	1.94×10^5
Stored in UK 5 yrs.	6.64×10^5	2.10×10^5
Stored in Khartoum-Sudan 5 yrs.	7.92×10^5	2.31×10^5
Stored in Port-Sudan 5 yrs.	8.45×10^5	2.63×10^5

As it can be observed from Table 6-1 all the stored samples show an increase in M_w value compared to the fresh sample. Samples stored in Port-Sudan and Khartoum-Sudan showed the highest increase in M_w which is approximately 42% and 33% respectively. The variation between the M_w values for the fresh and stored samples is statistically significant ($F(4,215) = 5.126$, p 0.01). Post-hoc Tukey test (used in ANOVA analysis to find the relation between the subgroup) revealed that the variation between M_w value of fresh samples and the stored samples in UK for 3 and 5 years is statistically not significant. However, the variations between M_w value for the fresh sample and stored samples in Port-

Sudan and Khartoum-Sudan are statistically significant. Also, the differences in M_w between samples stored in UK for 3 and 5 years and samples stored in Port-Sudan and Khartoum-Sudan for 5 years is statistically significant as shown in Figure 6.2.

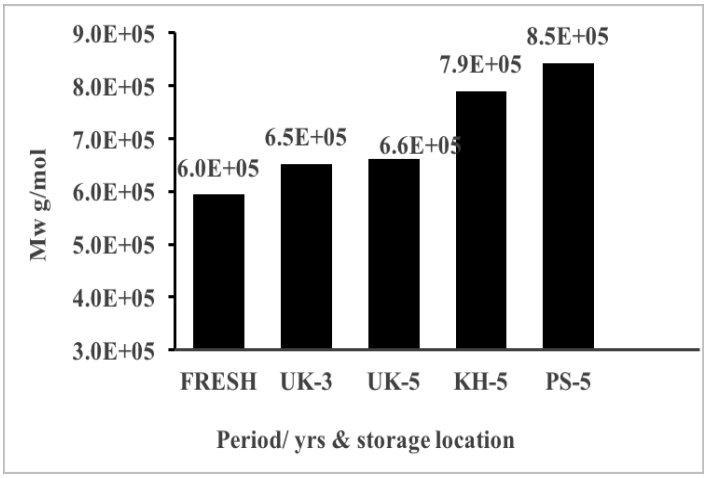


Figure - 6.2 Comparison between the mean M_w value for fresh samples and stored samples. Fresh-UK means the fresh sample examined in UK, UK-3 means sample stored in UK for years, UK-5 means sample stored in UK for 5 years, Kh-5 means sample stored in Khartoum-Sudan for 5 years and PS-5 means sample stored in Port-Sudan for 5years.

Table. 6-2 Comparison between the mean %AGP for fresh samples and stored samples.

Sample details	%AGP	SD
Fresh	13.6	4.51
Stored in UK 3 yrs.	15.2	4.84
Stored in UK 5 yrs.	15.8	5.01
Stored in Khartoum-Sudan 5 yrs.	16.1	5.06
Stored in Port-Sudan 5 yrs.	19.1	5.14

From Table 6-2 it is clear that all stored samples showed an increase in %AGP compared to the fresh sample as function of time. There is remarkably high increase in %AGP for samples stored in Port-Sudan and Khartoum-Sudan, the increase is approximately equal to

40% and 20 % respectively. The difference of %AGP between fresh and stored samples is statistically significant (F (4,215) = 2.759, p 0.029). Furthermore, using Post-hoc Tukey test revealed that only the variation between Port-Sudan stored sample and fresh samples is statistically significant (p 0.046) shown in Figure 6.3.

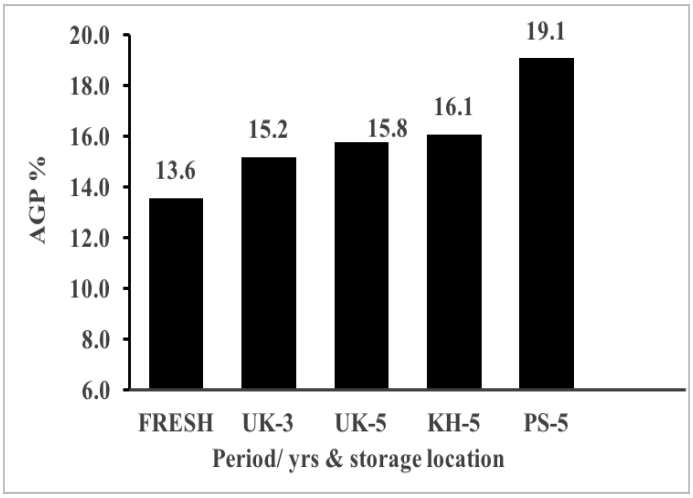


Figure - 6.3 Comparison between the mean %AGP for fresh samples and stored samples.

This result confirmed that both temperatures and humidity are very essential in the maturation process whether it conducted naturally or in the laboratory. This result is in a good agreement with the recent study by Al-Assaf et al (2012) where they achieved greater increase in M_w and %AGP by exposed the sample to constant temperature and humidity (Al-assaf, Andres-Brul, Cirre et al., 2012).

The interpretation of the above results is

(i) In UK, stored sample for 3 and 5 years, the natural maturation process is not stimulated to the limit that resulted in significant difference between M_w and %AGP. This can be explained due to the cold weather and low temperatures in UK.

(ii) The stored samples in Port-Sudan and Khartoum-Sudan for five years, the natural maturation process stimulated to the limit that resulted in significant variation in M_w value and %AGP. The factor of the high temperature in the both areas in-addition to the humidity factor in Port-Sudan, are very essential factors to induce the association of the small molecular weight arabinogalactan (AG) and glycoprotein (GP) unites into large units which taken a further stage to give larger molecular weight arabinogalactan protein (AGP) aggregates.

The new matured gum is chemically and molecularly identical to the fresh gum, but because of the different in distribution of smaller units into larger aggregates, the physical and functional performance is greatly enhanced. Illustrate in Figure 6.4. This out-come, is very much agreed with the patent of Hayashi 2002 which was achieved by the enhancement of gum arabic under the atmosphere of 30-100% of relative humidity at over 40 ℃ (Hayashi, 2002). It is also agreed with the finding of the recent study carried by Al-Assaf et al 2012 where, they investigated the effect of the storage condition on a fresh gum nodules; they observed greater increase in the M_w and %AGP for sample stored at constant temperature and humidity (Al-assaf et al., 2012). Notable, that the annual average of humidity and temperature in Khartoum-Sudan and Port-Sudan is extremely high compared to UK.

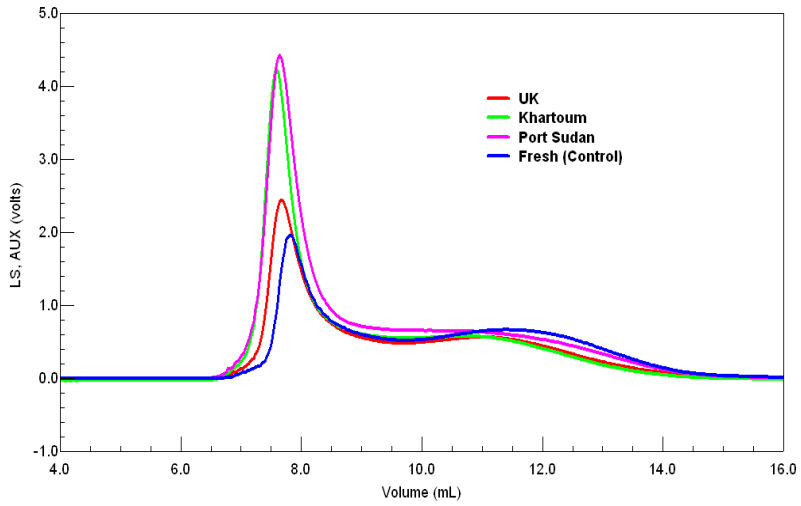


Figure - 6.4 Comparison of light scattering response for fresh and stored samples on molecular distribution peak 1 AGP showing the increase of AGP mass in stored samples.

Moreover, the synergistic impact of the storage condition in different locations with the effect of other factors such as, molecular weight level of samples, different age of the trees, picking set, samples location (East or West region) were also examined. The objectives here are to identify which of those factors contributed significantly in the natural maturation in acacia gum and therefore, caused changes in the structure and physiochemical properties. Figure 6.5 displayed the impact of the storage condition in different locations, on samples with different level of molecular weight value (M_w) high, medium and low. It is clear that the effect of storage condition is very significant in the samples that initially has high molecular weight (F (411) = 2.96, p 0.034). This phenomenon could be explained due to the high availability of the small molecule units in high molecular weight sample which could be easily aggregate together under heat and humidity to form larger molecular units. While the samples that has medium and low molecular weight showed the same trend however, the change is statistically not significant.

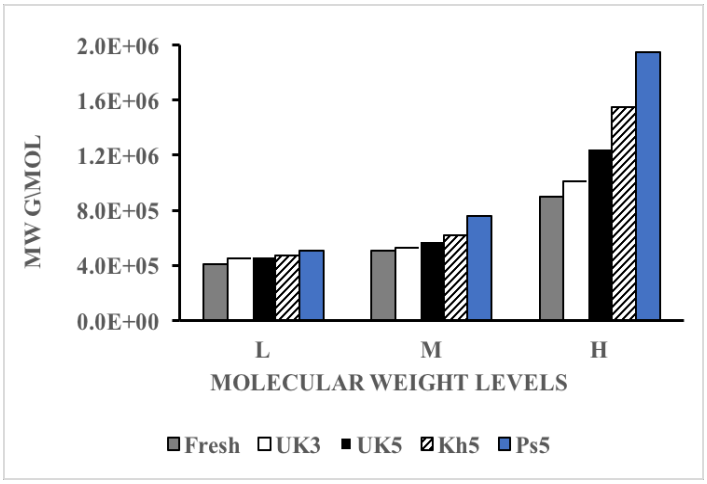


Figure - 6.5 The effect of storage condition on samples with different level of Mw L means low molecular weight level > 5.0 x 10^5 , M means medium molecular weight <5.0 x 10^5 > 7.0 x 10^5 and H means high molecular weight < 7.0 x 10^5.

The impact of storage condition on samples collected from different regions was also examined. Figure 6.6 shows the comparison of the effect of storage condition on samples collected from the west and east region in Sudan. The effect is evident in both regions and show the same trend. Samples stored in UK showed no significant increase in molecular weight value while a significant increase observed for those stored in Sudan-Khartoum and Port-Sudan. However, the trend in the west region is clearer than the east region and showed very significant increase in the M_w value which indicated that the natural maturation process is stimulated to a higher limit compared to the samples collected from the east region. This could be explained by the differences in the moisture content of the samples in the two region where the average of the moisture content for the fresh sample is 12.1% and 13.5% for samples originate from east and west region respectively, also in addition to the quick loss of the moisture for sample of eastern region compared to the sample from western region where the average of the moisture content after the period of the storage is 10.8% and 12.6% for east and west region respectively. Therefore, moisture content is linked to the availability of water inside the gum lumps and this give the small molecule more

freedom to freely move and associate together when stimulate by external factors such as heat and humidity to form larger molecule.

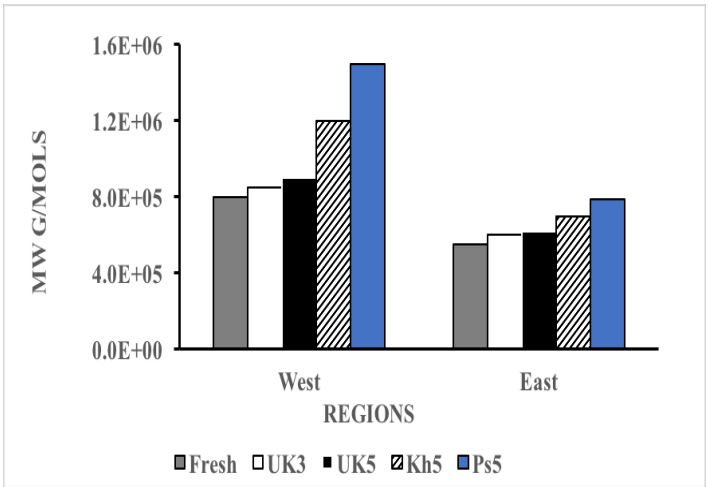


Figure - 6.6 The effect of storage condition on samples from West and East regions in Sudan.

Figure 6.7 shows the effect of the storage condition on the molecular weight value for samples collected from different ages of tree. The effect of storage condition is very limited in samples obtained from small trees age (5 and10 years) and old trees age (20 years), however, the effect of storage condition is much clear in samples that obtained from age of tree that considered as the peak of maturation of *Acacia* tree growth (15 years), this could be interpreted due to tendency of the samples that obtained from this age to the natural maturation process, since it has been reported that the growth of the trees also stimulate the maturation process (Al-Assaf et al., 2007). It has been also reported by Idris et al (Idris et al., 1998) that, the acacia trees reached the peak of growth maturation and give the highest molecular weight at age of 15 years. So, the combination factors of trees growth and storage condition could be responsible for this slightly increase in molecular weight value.

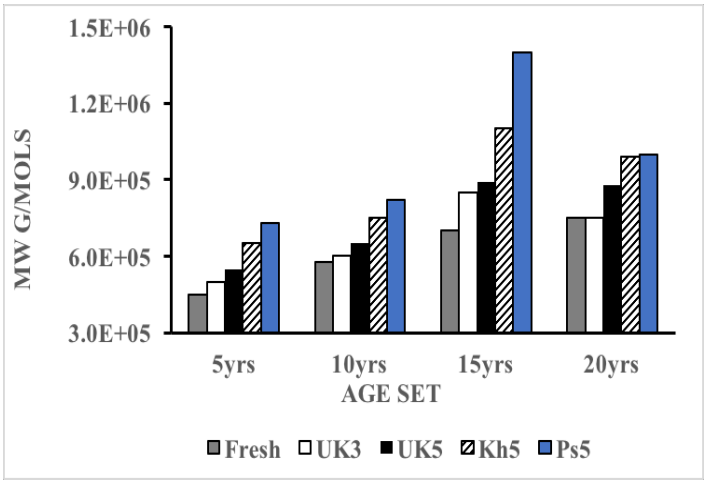


Figure - 6.7 The effect of storage condition on samples from different ages of tree.

6.1 Conclusion

The natural maturation in *A. senegal* gum can result in very significant change in the structure and therefore, in the physiochemical properties and functional properties. The maturation is physically achievable by storage the gum for certain period of time, with the presence of hot temperature and humidity. In this study, samples which were stored in Khartoum-Sudan (hot and dry) and in Port-Sudan (hot and humid) revealed significant change in M_w 33% and 42 % respectively and also significant increase up to 20% and 40% in AGP mass compared to the initial value from the fresh sample. On the other hand, samples stored in UK (cold and humid) shown no significant change in M_w value and AGP mass. This finding further demonstrated that the temperature and humidity combined together are essential factors that play the main role to stimulate the natural maturation process. The presence of one factor without the other is not enough to achieve a significant change.

Moreover, samples that initially has high molecular weight are much capable to attain significant change through the natural maturation process compared to low and medium molecular weight samples. The natural maturation process is more likely for sample

originally from west region in Sudan after storage for period of time, the change in the properties in particularly the changes in M_w and AGP mass are very remarkable compared to samples originally from the east region in Sudan.

Chapter 7

7. Evaluation of the emulsification performance

In this chapter, the emulsification performance of gum arabic samples used in this study was chosen as a measure of functionality. As described already (section 1.7) Gum arabic ability to emulsify oil in water emulsion has been well studied (Al-Assaf et al., 2007; Castellani, Gaillard, Vié et al., 2010; Dickinson, 2003). It has been widely accepted now that the proportion of the arabinogalactan-protein fraction (AGP) is mainly responsible for conferring the emulsification performance and stability of Gum arabic. All samples used in this study were evaluated using a model beverage emulsion recipe namely: 20% Gum arabic, 20% MCT oil, 0.12% citric acid and 0.13 % sodium benzoate as described in section (3.2.6.2.1). The measurement of the droplets size and distribution was carried out using laser diffraction (see section 3.2.6.2.2).

Emulsification efficacy is usually evaluated based on the initial particle size which is a valuable indicator of quality and performance. Subsequently, the emulsions were subjected to an accelerated stress testing (3 and 7 days' incubation at 60 ^{o}C) and the droplet size distribution was measured again. The incremental changes in the droplet size distribution was taken as a measure of emulsion stability. The emulsion index was calculated according to the following equation 7-1.

Emulsion Index = Initial VMD + (Δ after 3 days- micron) + (Δ after 7 days micron). [7-1]

Example of emulsion VMD index calculation given in table 7.1which showed representative examples of different emulisfer strength (good, medium and poor).

Table 7-1. An example for three set of results of MVD and Index calculation representating 168 samples used in this study.

VMD (μm)				
Initial	3 days at 60°C	7 days at 60°C	VMD Index	Emulsion strenght
0.261	0.393	0.423	0.455	Good
0.258	0.375	0.603	0.72	Medium
0.281	0.437	0.807	0.96	Poor

The Index –VMD number is indicator for the emulsifier strength where, <0.7 is good emulsifier, 0.7 – 0.85 medium emulsifier and > 0.85 is poor emulsifier. Comparison between the examples of the different emulsifiers strength shown in Figure 7.1a and 7.1b using VMD and volume (%) of droplet size greater than 1 micron.

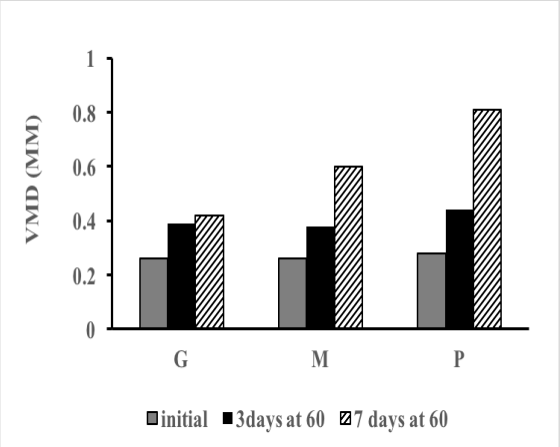


Figure - 7.1a

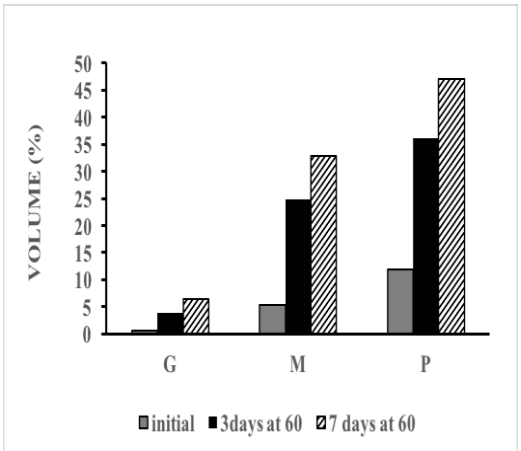


Figure - 7.1b

Figure 7.4a and **7.4b** Show a comparison between different emulsifiers using VMD and > 1 μm for the same sample respectively. G = good emulsifier, M= medium emulsifier and P = poor emulsifier.

The 168 samples used in this study were all evaluated for emulsification performance and stability. A uniform narrow distribution was obtained for emulsions classed as good emulsifiers (Figure 7.2) where all droplets are below 1 micron. Emulsions classed as an average gave typical droplet size distribution as shown in Figure 7.3 where a shoulder, to a varying extent, appeared with droplets bigger than 1 micron. Poor emulsifiers gave larger proportion of droplets higher than 1 micron and showed either an extended shoulder or a bimodal distribution (Figure 7.4).

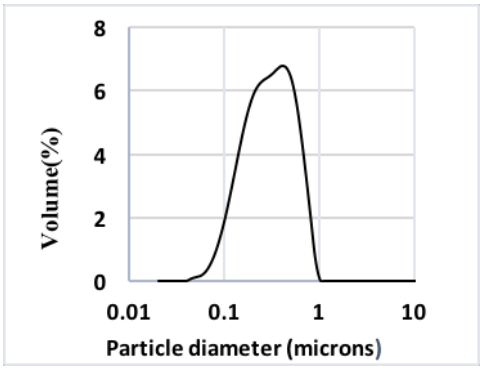


Figure - 7.2. An example of small droplets size distribution of an oil in water fresh emulsion (O/W) made using Gum arabic: MCT oil at 1:1 ratio.

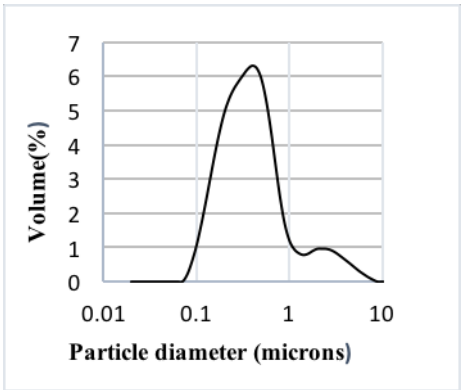


Figure - 7.3. An example of medium droplets size distribution (O/W) fresh emulsion.

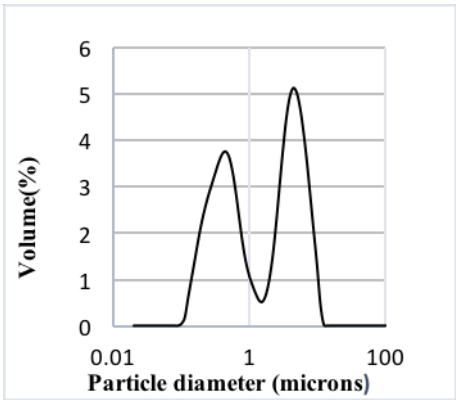


Figure - 7.4. An Example of large droplets size distribution (O/W) fresh emulsion.

The layer of *Acacia* gum that adsorbs on the surface of oil droplets influences the density of the droplets. The amount of adsorption for each volume changes depending on the droplet size. Generally, when the droplet size grows, the density of droplet approach the density of original oil which is lighter than the density of water phase and that cause the creaming layer on the surface of the emulsion, therefore, the volume changes. Therefore, the value of the particles size which divides the population into exactly two equal halves, i.e. there is 50% of the distribution above this value and 50% below known as volume median diameter (VMD) is used in this study as measure of emulsion stability. Moreover, the rate of creaming is related to the square of the droplet radius according to Stokes Law (see section 1.7). So, large droplets often cause a creaming problem. In this study, the percentage of large droplets (i.e. > 1 μm) generated is also used to evaluate the emulsion stability.

Data of the volume median diameter (VMD) and changes in % volume of droplets greater than 1 micron obtained from the emulsion tests of all 168 samples were subjected to a one-way analysis of variance (ANOVA), correlation and linear regression test. The statistical analysis was performed at P-value α = 5% as described in section 3.2.7. As already concluded in Chapter 5 that (i) no significant differences between the specific and representative samples, (ii) no significant differences between the results of plantations

within the same region and the significant variation is only observed between the two regions. Therefore, in this section, all the samples were used and subjected to the statistical analysis to evaluate the emulsification performance and the result compared base on west and east region.

7.1 Influence of the physiochemical properties on the emulsification performance

The objective of this section is to determine the influence of the physiochemical properties of Gum arabic samples, which vary widely depending on Location (West or East) on the emulsification performance. The influence of the % protein, viscosity, M_w and % AGP will be examined as described above.

The statistical regression analysis for initial VMD for the samples (represented in Table 7-1) showed negative correlation with % protein, intrinsic viscosity, M_w and % AGP, however those correlations are statistically not significant (r = -0.1, -0.02, -0.01 and -0.1) respectively. The significant regression equation was found (F (4,155) = 1.593, p n.s), with an $r^2$of 0.039. The regression model is not significant and only 3% of total variation in VMD of initial droplet is dependent on the above mentioned physiochemical properties. This indicates that the influence of these properties combined on emulsion ability is not significant and also separately did not significantly influence the initial VMD. Furthermore, the multi regression analysis for the VMD obtained from the acceleration test (3 and 7 days at 60 C°) revealed strong negative correlation between the changes in VMD and the physiochemical properties, in particular the % AGP (r =0.60), viscosity (r = 0.51), M_w (r = 0.41) and protein% (r = 0.25). The multiple linear regression was calculated to predict VMD droplet after the acceleration test based on % protein, viscosity, M_w and % AGP. A significant regression equation was found (F (4,155) =12.604, p 0.000), with an $r^2$of 0.326. The regression model for prediction of VMD droplet after acceleration is very significant.

33% of variation on VMD droplet after acceleration (which is indicator to emulsion stability) can be explained by the above-mentioned properties. The physiochemical properties predicted VMD droplet after acceleration test as in following equation:

VMD after acceleration =2.545- 0.163(protein) - 0.058(viscosity) -1.25 E-7(Mw)– 0.015(AGP). This means that the participants VMD after acceleration decreased by 0.163 micron for each 1% of protein, decreased 0.058 micron for each g/cm3 of viscosity, decreased 1.25 E -7 for each 1 g/mol of M_w and decreased 0.015 for each 1% of AGP. Only % AGP and viscosity were significant predictors (p 0.001 and p 0.033) of VMD droplet after acceleration test. Therefore, both can be used as predictors of emulsion stability.

From the correlation and multiple linear regression analysis, it is clear that % AGP is the most influential factor and has strong positive correlation with *A. senegal* gum emulsion stability followed by viscosity. These results are in good agreement with previous studies which concluded that the emulsifying properties of *A. senegal* gum not only depend on the total of protein content but it also on its distribution between the low and high molecular weight fractions (Al-Assaf et al., 2007; Aoki, Al-Assaf, Katayama et al., 2007; Buffo et al., 2001; Castellani, Gaillard, et al., 2010).

7.2 The influence of different picks on the emulsification performance

7.2.1 Evaluation of initial droplet for samples from different picks

The emulsification performance of *A. senegal* samples collected from different picks was also evaluated. The mean of the initial volume median diameters (VMD) in both eastern and western regions for samples obtained from 1st, 2nd, 3rd and 4th picks respectively are tabulated in Table 7-2 and 7-3. The mean of initial VMD was statistically different in both regions as a function for different picks, (F (3,64) =5.459, p 0.002) and (F (3,92) = 2.811, p 0.04) for east and west region respectively. The multiple comparison test (Tukey HSD)

showed that the mean of the initial VMD for the 1st and 2nd picks had significantly lower value than the 3rd and 4th picks. Thus, the emulsification efficiency for gum arabic collected from 1st and 2nd picks is significantly higher than gum collected from 3rd and 4th picks. Furthermore, the emulsification performance was also evaluated by using the % volume of droplets greater than 1 µm. The descriptive statistics associated with this mean across the different picks in eastern region were 1.83 µm, 1.50 µm, 2.70 µm and 1.95 µm for samples obtained from 1st, 2nd, 3rd and 4th picks respectively and in western region were 0.83 µm, 2.0 µm, 1.30 µm and 0.67 µm for samples obtained from 1st, 2nd, 3rd and 4th picks respectively. The mean % volume of droplets above 1 µm was not statistically different in both regions as a function of different picks ($F(3,64) = 0.535$, p n.s) and ($F(3,92) = 1.696$, p n.s) for east and west region respectively. The results of VMD showed statistically significant differences in emulsification ability as function of different picks. This could be explained due to the exist of high % AGP in the interface layer of oil and water in emulsions for samples obtained from 1st and 2nd picks. Which formed elastic film layer prevent the initial droplets from breakage and coalescence. However, the result of % volume of droplets above 1 µm showed that different picks of *A. senegal* gum had no significant effect on the initial emulsification ability.

7.2.2 Accelerated stability test of emulsions correlation with different picks

The descriptive statistic associated with the change in the mean value of VMD after acceleration test (3 and 7 days' incubation at 60 °C) across the 4 different picks in the western and eastern regions are tabulated in Table.7-2 and 7-3. It can be seen that samples obtained from the 1st and 2nd pick in the both region had the highest emulsion stability. This could be explained due to the highest value % AGP in the 1st and 2nd pick as reported in (section 5.2). The changes in the mean VMD after the acceleration test was significantly

different after 3 days at 60 °C in the eastern region only (F (3,64) = 4.34, p 0.008) and it was also significantly different with correlation with the different pick in the both regions after 7 days at 60 °C. (F (3,64) = 9.811, p 0.00002) and (F (3,92) = 16.56, p 0.00000003) for east region and west region respectively. A visual depiction of the changes in the mean value of VMD after acceleration test with correlation of different picks in both region is shown in Figure 7.5 and 7.6. Post – hoc Tukey HSD test for multiple comparison showed that in the both regions the emulsion of samples collected from 1st and 2nd pick had significantly less changes in VMD after the acceleration test compared with the samples collected from 3rd and 4th pick.

This result revealed that the stability of emulsion obtained from samples collected from 1st and 2nd pick significantly higher than emulsion form from samples collected from 3rd and 4th pick. Moreover, emulsion of samples collected from 1st and 2nd picks in western region tend to show higher stability than emulsion obtained from the same picks in western region which showed significant changes in VMD after 3 days' incubation at 60 °C and this not the case in the western region.

Table 7-2. Change in volume median diameter (VMD) of samples collected from eastern region from different picks during storage for 3 and 7 days at 60 °C.

	VMD (μm)		
Pick set	**Initial**	**3days at 60 °C**	**7days at 60 °C**
1st Pick	**0.334**	**0.358**	**0.387**
SD	*0.05*	*0.06*	*0.08*
2nd Pick	**0.328**	**0.357**	**0.410**
SD	*0.04*	*0.06*	*0.09*
3rd Pick	**0.293**	**0.385**	**0.452**
SD	*0.05*	*0.07*	*0.10*
4th Pick	**0.351**	**0.452**	**0.637**
SD	*0.05*	*0.09*	*0.20*

Table 7-3. Change in volume median diameter (VMD) of samples collected from western region from different picks during storage for 3 and 7 days at 60 °C.

	VMD (μm)		
Pick set	**Initial**	**3days at 60 °C**	**7days at 60 °C**
1st Pick	**0.302**	**0.324**	**0.333**
SD	*0.1*	*0.12*	*0.06*
2nd Pick	**0.318**	**0.345**	**0.336**
SD	*0.05*	*0.06*	*0.06*
3rd Pick	**0.319**	**0.391**	**0.478**
SD	*0.05*	*0.11*	*0.08*
4th Pick	**0.367**	**0.399**	**0.465**
SD	*0.05*	*0.05*	*0.05*

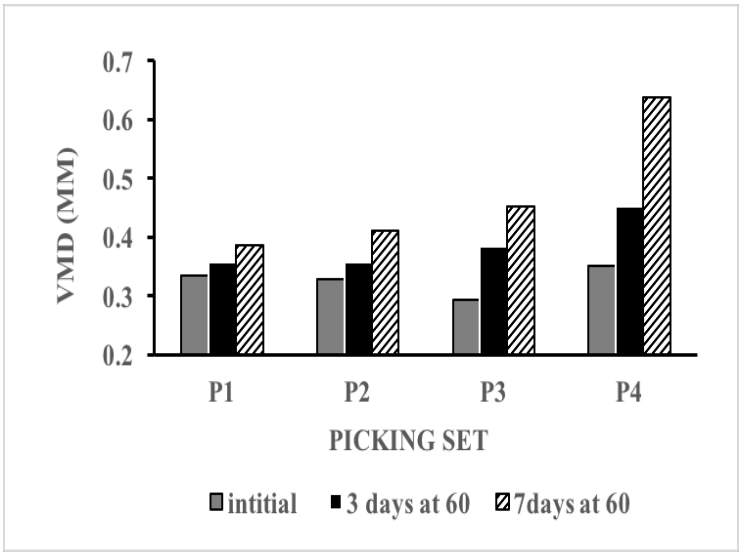


Figure - 7.5. Changes in VMD for samples collected from eastern region of different picks emulsions by accelerated test.

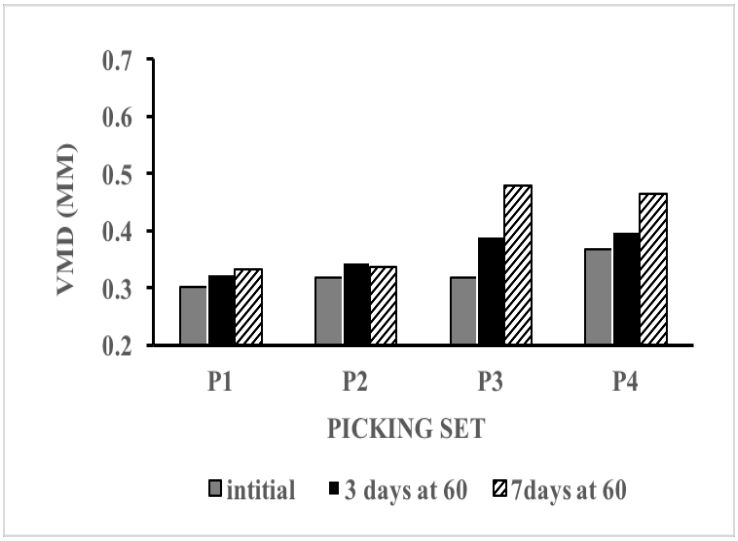


Figure - 7.6. Changes in VMD for samples collected from western region of different picks emulsions by accelerated test.

Moreover, the ratio of large droplets generated during the acceleration test was also used as another parameter for the evaluation of emulsion stability in both regions. The results are listed in Table 7-4 and 7-5 and shown in Figure 7.7 and 7.8. The results in both eastern and western regions demonstrated that the deterioration (destabilisation) of droplets size for emulsion samples from the first picks is very small and increases thereafter up to the fourth picks. This deterioration was caused by the collision of the droplets which increase frequently due to the higher temperature of the accelerated test, therefore led to coalescence of the emulsion. Nevertheless, the sufficient ability of the elastic film layer which formed by high molecule weight fraction (AGP) in *Acacia* gum can prevent this coalescence. The variation in the deterioration of the droplets size as function for different picks after 3 days incubation at 60 °C was only statistically significant in the eastern region. ($F(3,64) = 4.976$, $p\ 0.004$). Additionally, the variation in the deterioration of the droplets size as function for different picks after 7 days' incubation at 60 °C was statistically significant in both east and west region ($F(3,64) = 4.881$, $p\ 0.004$) and ($F(3,92) = 4.914$, $p\ 0.003$) respectively. Post – hoc Tukey HSD in the both region confirmed the significant difference in emulsion stability of samples from 1st and 2nd picks compared with 4th pick.

Both results of MVD and % volume of droplets above 1 µm suggest that different picks of *A. senegal* gum had a significant effect on the emulsion stability. Thus, null hypothesis of no differences between the emulsion stability for samples collected from different picks was rejected.

Table 7-4. Change in above 1 µm volume (%) of samples from eastern region from different picks emulsions by accelerated stability test.

	volume % of droplets above 1 μm		
Pick set	**Initial**	**3days at 60 ºC**	**7days at 60 ºC**
1st Pick	**2.2**	**8.10**	**20.3**
SD	*0.93*	*3.00*	*6.89*
2nd Pick	**2.5**	**10.41**	**21.0**
SD	*1.3*	*4.02*	*8.85*
3rd Pick	**3.10**	**14.96**	**30.21**
SD	*1.8*	*9.0*	*11.75*
4th Pick	**2.54**	**25.52**	**46.62**
SD	*1.4*	*12.98*	*18.68*

Table 7-5. Change in above 1 µm volume (%) of samples from western region from different picks emulsions by accelerated stability test.

	volume % of droplets above 1 μm		
Pick set	**Initial**	**3days at 60 ºC**	**7days at 60 ºC**
1st Pick	**0.833**	**3.76**	**7.39**
SD	*0.63*	*1.00*	*4.89*
2nd Pick	**2.0**	**5.02**	**7.62**
SD	*1.2*	*2.01*	*3.85*
3rd Pick	**1.30**	**5.23**	**11.55**
SD	*1.1*	*3.7*	*6.75*
4th Pick	**0.67**	**5.55**	**14.94**
SD	*0.4*	*1.98*	*7.68*

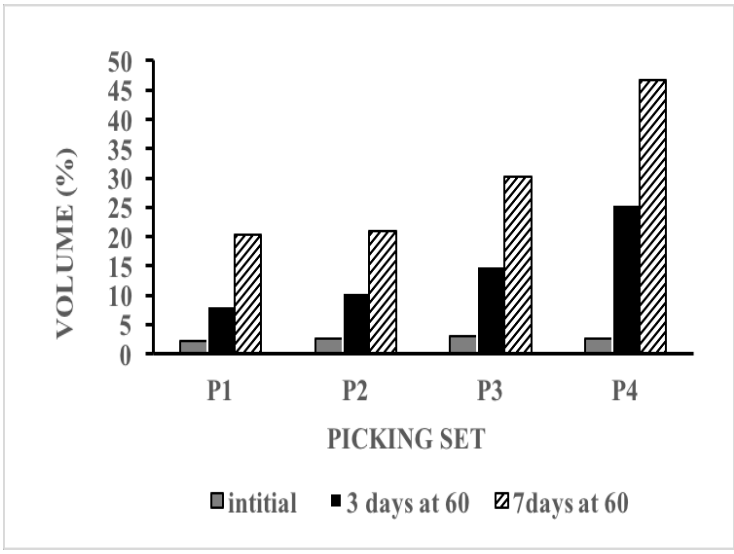


Figure - 7.7. Change in above 1 μm volume (%) of samples from eastern region from different picks emulsions by accelerated stability test.

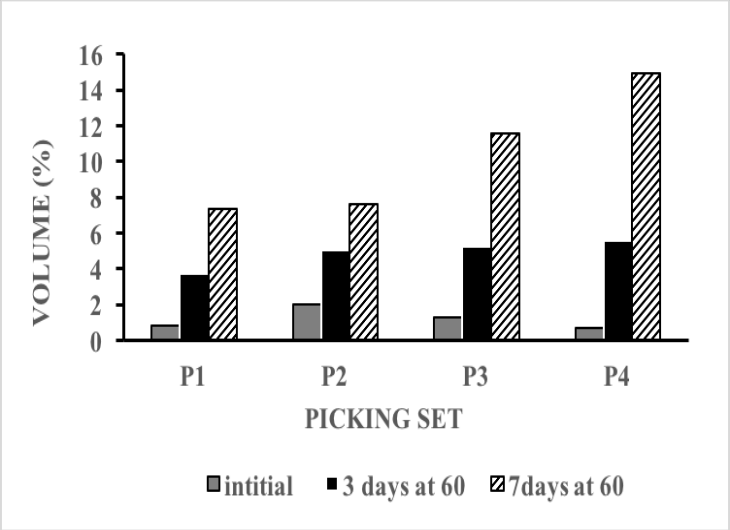


Figure - 7.8. Change in above 1 μm volume (%) of samples from western region from different picks emulsions by accelerated stability test.

7.3 The influence of tree age on the emulsification performance

7.3.1 Evaluation of initial droplet for samples from different tree age set

In this section, the emulsification performance for *A. senegal* samples collected from four different age set, was evaluated using ANOVA one-way analysis in both eastern and western regions. The descriptive statistic correlating with the mean values of the initial VMD across the four-different age sets in eastern region were 0.299 (0.05) μm, 0.292 (0.04) μm, 0.285 (0.05) μm and 0.330 (0.05) μm for samples collected from trees 5, 10, 15 and 20 years old respectively. In western region, the initial MVD were 0.333 (0.08) μm, 0.331 (0.05) μm, 0.301 (0.06) μm and 0.314 (0.06) μm for samples collected from trees 5,10, 15 and 20 years old respectively. As it can be observed that the smallest mean value of VMD in both region was obtained from the 15 years old tree. However, the differences between the initial mean of VMD for samples collected from different tree ages in both regions was statistically not significant ($F(3,64) = 2.09$, $p\ 0.11$) and ($F(3,92) = 2.202$, $p\ 0.093$) for eastern and western regions respectively.

Moreover, the emulsification ability was also evaluated by using % volume of droplet above 1μm. The mean results on initial droplets in eastern region were 1.94 (1.2) %, 2.13 (1.1) %, 1.84 (1.06) % and 2.14 (1.09) % for samples collected from trees 5, 10, 15 and 20 years old respectively. In the western region, the mean value of initial droplets was 1.15 (0.089) %, 0.815 (0.15) %, 0.762 (1.6) % and 1.314 (0.92) % for samples collected from trees 5, 10, 15 and 20 years old respectively. The lowest volume % of droplet above 1 μm was observed in samples collected from 15 years old tree in the both regions. The variation between the mean volume % of droplet above 1 μm across sample from the different ages of trees was statistically not significant in both regions ($F(3,64) = 0.04$, $p\ 0.989$) and ($F(3,92) = 0.197$, $p\ 0.946$. Both results of VMD and volume % above 1 μm suggest that the age of the tree in both regions did not have significant influence in the emulsification ability.

7.3.2 Accelerated stability test of emulsions for sample from ages of tree

ANOVA one-way analysis was also performed to compare the impact of the age of the *A.senegal* trees on the emulsion stability for samples collected from the both eastern and western region. Samples in the both regions were divided into four groups based upon their age (5 years, 10 years, 15 years and 20 years). The descriptive statistic associated with the changes VMD value across the four different groups of ages after acceleration test in the east and west region are reported in Table 7-6 and 7-7. The less changes in VMD were obtained from samples collected from 15 years old in the both regions. However, there was no statistically significant difference in VMD mean scores for the four age groups in the both east and west regions ($F(3,64) = 0.147$, $p = 0.931$) and ($F(3,92) = 1.545$, p 0.208) respectively. The result shown in Figure 7.9 and 7.10.

Table 7-6. Change in volume median diameter (VMD) of samples collected from east region from different age set during storage for 3 and 7 days at 60 ºC.

	VMD (μm)		
Age set	**Initial**	**3days at 60 ºC**	**7days at 60 ºC**
5 years	**0.299**	**0.372**	**0.452**
SD	*0.05*	*0.08*	*0.14*
10 years	**0.292**	**0.367**	**0.449**
SD	*0.04*	*0.05*	*0.17*
15 years	**0.290**	**0.324**	**0.428**
SD	*0.05*	*0.09*	*0.15*
20 years	**0.330**	**0.415**	**0.434**
SD	*0.05*	*0.05*	*0.05*

Table 7-7. Change in volume median diameter (VMD) of samples collected from west region from different age set during storage for 3 and 7 days at 60 ºC.

	VMD (μm)		
Age set	**Initial**	**3days at 60 ºC**	**7days at 60 ºC**
5 years	**0.333**	**0.398**	**0.402**
SD	*0.08*	*0.12*	*0.09*
10 years	**0.331**	**0.383**	**0.418**
SD	*0.05*	*0.06*	*0.07*
15 years	**0.303**	**0.398**	**0.395**
SD	*0.06*	*0.06*	*0.06*
20 years	**0.314**	**0.375**	**0.370**
SD	*0.06*	*0.09*	*0.08*

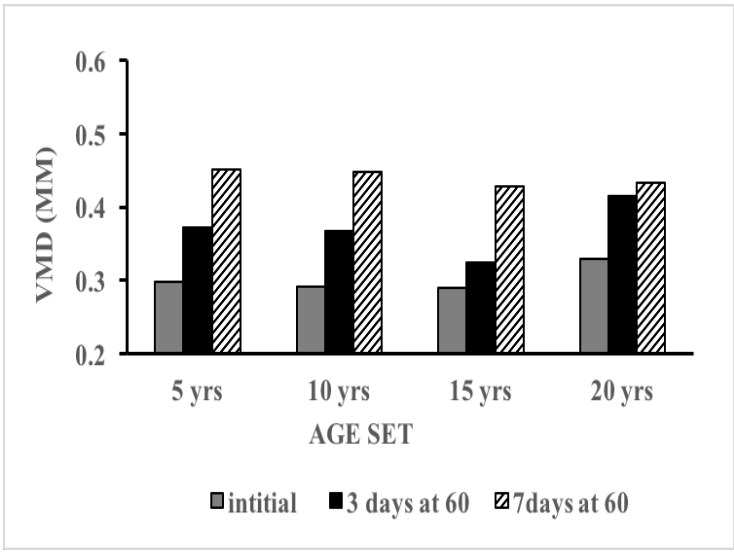


Figure - 7.9. Change in VMD for samples collected from eastern region of different age set emulsions by accelerated test.

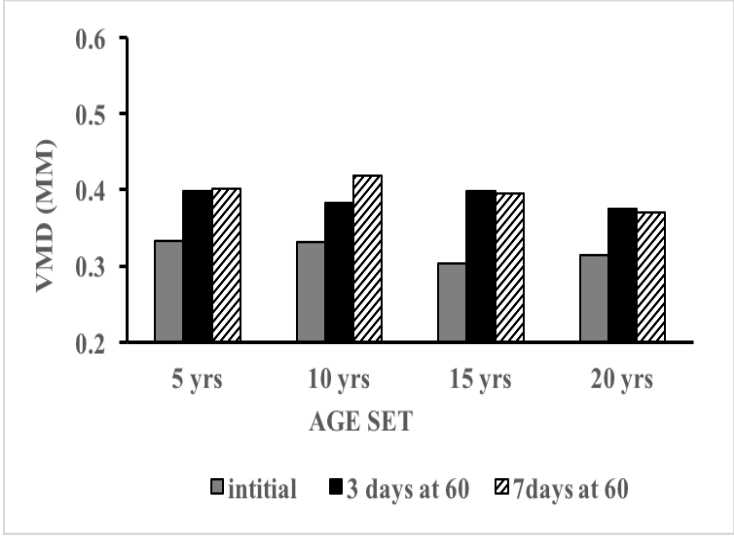


Figure - 7.10. Change in VMD for samples collected from western region of different age set emulsions by accelerated test.

Furthermore, the contribution of different groups of trees age (5, 10, 15 and 20 years) to the emulsion stability was also examined in the both regions using the % volume of droplet

above 1µm parameter. The descriptive statistic associated with the changes in % volume of droplet above 1µm across the four different groups of ages after acceleration test in the east and west region are given in Table 7-8 and 7-9. As it can be seen the less change in % volume of droplets above 1 µm after accelerated test was obtained from samples collected from 15 years old in both east and west region. However, samples collected from the western region showed significantly less change in % volume of droplets above 1µm after accelerated through all age set compared to the samples collected from eastern region. There was no statistically significant difference in the mean values of the volume (%) of droplet above 1µm as a function for different tree ages in the eastern region (F (3,64) = 0.123, p 0.946) shown in Figure 7.11. While, in the west region there was statistically significant in the mean values of the volume (%) of droplet above 1µm as a function for different tree ages (F (3,92) = 4.142, p 0.008). Applied Post-hoc Tukey HSD test for the result in west region showed that the mean volume (%) of droplet above 1µm for a group of 15 and 20 years old tree has the lowest changes after the acceleration test 7 days' incubation at 60 °C (mean= 6.926%, SD=3.11) and (mean = 7.37%, SD = 4.21) respectively. They were significantly different from groups of ages (5 years and 10 years) shown in Figure 7.12.

Table 7-8. Change in above 1 μm volume (%) of samples from eastern region from different age set emulsions by accelerated stability test.

	% volume of droplets above 1 μm		
Age set	**Initial**	**3days at 60 °C**	**7days at 60 °C**
5 years	**1.94**	**13.51**	**26.80**
SD	*1.2*	*7.02*	*10.4*
10 years	**2.13**	**13.18**	**26.34**
SD	*1.19*	*7.84*	*17.18*
15 years	**1.84**	**9.92**	**23.43**
SD	*1.06*	*6.34*	*11.9*
20 years	**2.14**	**11.96**	**27.51**
SD	*1.09*	*6.02*	*13.6*

Table 7-9. Change in above 1 μm volume (%) of samples from western region from different age set emulsions by accelerated stability test.

	% volume of droplets above 1 μm		
Age set	**Initial**	**3days at 60 °C**	**7days at 60 °C**
5 years	**1.15**	**6.708**	**10.931**
SD	*0.089*	*4.35*	*7.36*
10 years	**0.815**	**7.705**	**14.984**
SD	*0.15*	*3.75*	*9.02*
15 years	**0.762**	**3.623**	**6.926**
SD	*0.67*	*1.36*	*3.11*
20 years	**1.314**	**1.535**	**7.37**
SD	*0921*	*1.31*	*4.21*

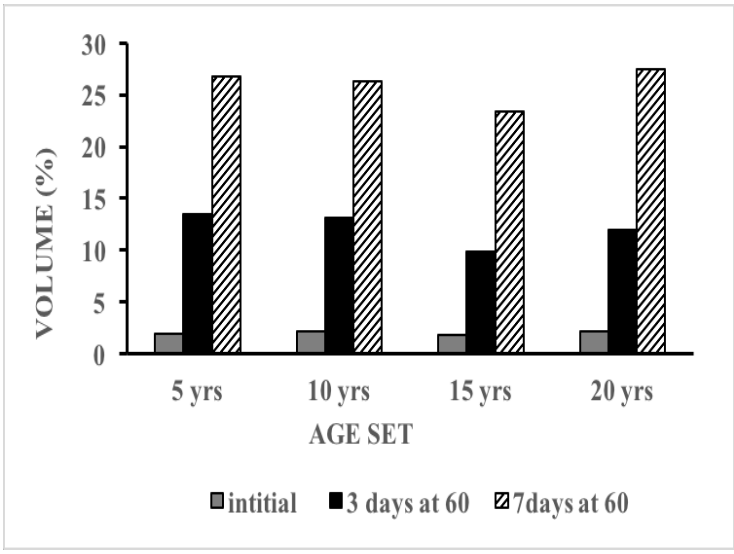


Figure - 7.11. Change in above 1 μm volume (%) of samples from eastern region from different trees age set emulsions by accelerated stability test.

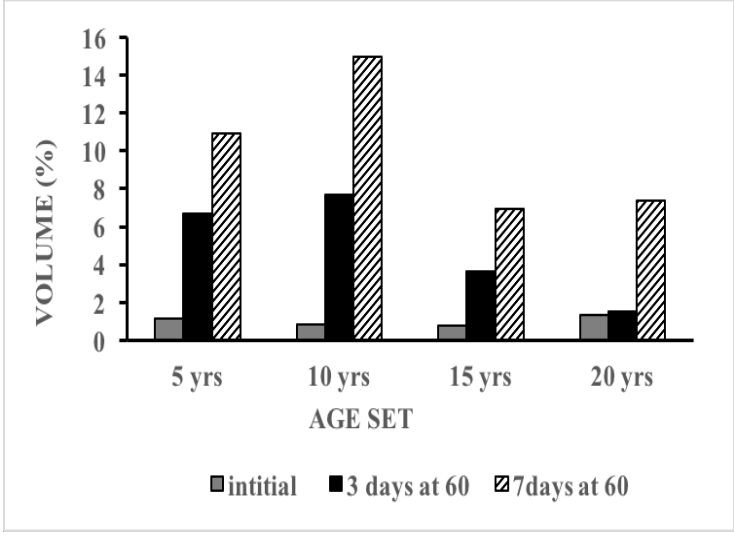


Figure - 7.12. Change in above 1 μm volume (%) of samples from western region from different trees age set emulsions by accelerated stability test.

7.4 The effect of storage conditions in emulsification performance

The objective of this section is to examine the impact of storage condition for all stored samples (mentioned in chapter 6) on the emulsification performance. As mentioned already, the impact of the storage conditions on the physiochemical properties such as % protein, viscosity, M_w, % AGP has been reported in this study (see Chapter 6). It was concluded that significant changes in the molecular weight and % AGP were evident in samples stored in Khartoum and Port-Sudan compared to those stored in the UK. The main factors responsible for this change are the temperature and humidity. An increase of ~20% in the above-mentioned parameters was obtained for samples stored in Khartoum whereas up to 40% for those stored in hot and humid environment such as Port Sudan. Of particular relevance is the % AGP increase which has already proven to have a direct effect on the emulsification performance as demonstrated above and widely reported (Al-Assaf et al., 2007; Castellani, Gaillard, et al., 2010; Dickinson, 2003).

The emulsification ability for the stored *A. senegal* gum samples (see section 3.1.4) was evaluated by ANOVA one-way analysis. Comparison between the mean of initial MVD across the fresh and the stored samples were used as emulsification ability evaluation mechanism. The descriptive statistic associated with initial VMD value across the fresh and the stored samples were 0.50 (0.12) μm, 0.46 (0.21) μm, 0.48 (0.13) μm and 0.39 (0.085) μm for fresh samples, UK stored samples, Khartoum stored samples and Port-Sudan stored samples respectively. The results show that the emulsification ability of all stored samples improved compared to the fresh samples. These results are consistent with the measurements reported in Chapter 6 and also in good agreement with the previous reports about the role of AGP proportion on the initial droplet size distribution of emulsions (Katayama et al, 2006). However, the improvement in the initial emulsification

performance for the stored samples compared with fresh samples was statistically not significant. (F (4,215) = 0.361, p n.s).

The emulsion stability of the stored samples was compared with the emulsion stability of the fresh samples by using ANOVA one-way analysis. The descriptive statistic associated with the changes in the mean of VMD after acceleration for fresh and the stored samples are summarized in Table 7-10. The results have shown an increase in VMD as a function of increasing the storage time at 60 °C. The stored samples showed a significantly less change in the VMD after acceleration compared to the fresh samples. The Post-hoc Tukey test revealed that sample stored in Port-Sudan had a significantly less changes in VMD after acceleration compared with the fresh sample, UK 3 years and 5 years stored sample at the 0.05 level of significance. However, it did not differ significantly compared to Khartoum stored samples. Thus, samples stored in Port-Sudan showed highest emulsion stability as shown in Figure 7.13.

Table 7-10. Changes in the mean of (VMD) for fresh and stored samples emulsion during storage 7 days at 60 C°.

	VMD (μm)		
Fresh & stored samples	**Initial**	**3 days at 60 C°**	**7 days at 60 C°**
Fresh samples	**0.50**	**0.58**	**0.64**
SD	*0.12*	*0.098*	*0.14*
UK stored samples	**0.46**	**0.50**	**0.61**
SD	*0.210*	*0.2*	*0.28*
Kh-stored samples	**0.48**	**0.48**	**0.54**
SD	*0.13*	*0.30*	*0.11*
PS-stored samples	**0.39**	**0.40**	**0.43**
SD	*0.086*	*0.0892*	*0.15*

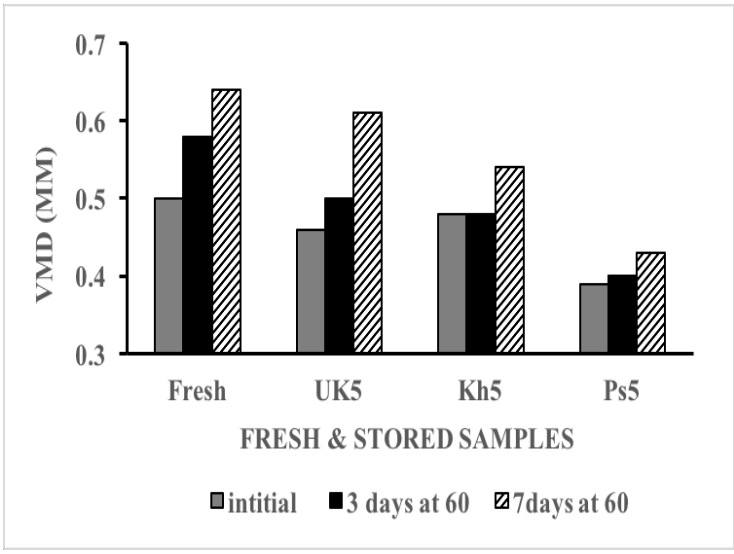


Figure - 7.13. Change in VMD for fresh samples and stored samples emulsions after accelerated test.

The results give above further confirm that temperature and humidity are the vital factors to induce the natural maturation process which led to increase in % AGP and therefore enhancement of the emulsion stability. This correlation between the emulsion stability for fresh and stored samples with the % AGP for fresh and stored samples is shown in Figure 7.14.

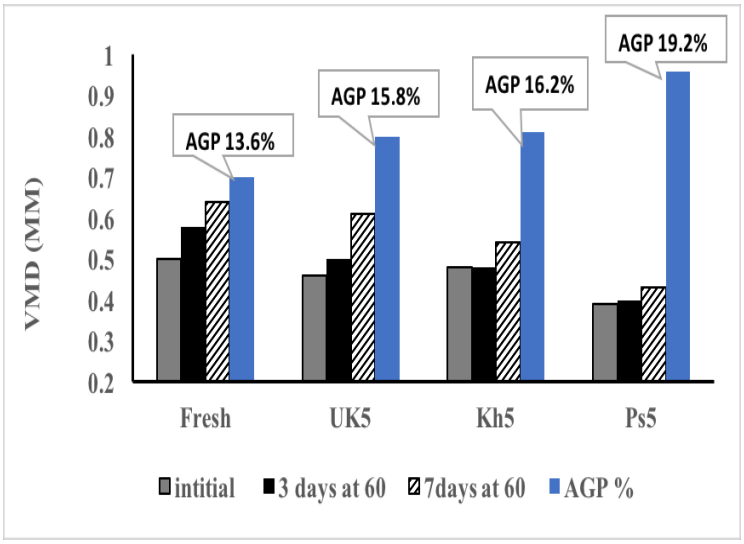


Figure - 7.14. Correlation between the emulsion stability for fresh and stored samples with the %AGP for fresh and stored samples.

7.5 Conclusion

The statistical regression analysis for the physiochemical properties which examined the correlation of these properties with the emulsification performance revealed that %AGP is the most influential factor for the emulsification performance followed by viscosity. % Protein showed no significant influence on the emulsification performance or stability. The variation present in %AGP due to the influence of various factors, such as, location, different picks, different ages of the tree and storage condition, does not cause any significant variation in the initial VMD. However, it caused significant variation in the initial % volume of droplets greater 1 micron.

Samples collected from plantations located in the west region in Sudan demonstrated the highest emulsification performance compared to those from the eastern region. This conclusion was mainly underpinned by the proportions of AGP fraction in the respective region. Moreover, the emulsification performance and stability of samples collected from the 1^{st} and 2^{nd} picks are significantly higher than those from 3^{rd} and 4^{th} picks. Furthermore, samples that obtained from a certain age of tree (15 years), displayed a superior emulsification performance compared to the samples collected from other ages of tree. Again, the main factor responsible for this performance was the high proportion of the AGP fraction.

Finally, storage of *A. senegal* for a period of five years in different locations resulted in an improvement of the emulsification performance. However, the most significant improvement in the emulsification performance was associated with locations that have high temperatures and humidity.

Chapter 8

8.1 Overall conclusions

The variations in the environmental factors (soil type, rainfall, humidity and temperature) between the two regions (East and West) in the gum belt in Sudan as well as the factors of collecting gum samples from different trees with varying age from 5-20 years and from different interval picks (1-4) were investigated in this study. The results clearly demonstrate how these factors can significantly influence the physiochemical and functional properties of *A. senegal* gums.

The results of 168 samples for all parameters examined in this study were in very good agreement with *A. senegal var senegal* specifications as set by JECFA (JECFA/FAO, 1997, JECFA-FAO, 1999) and those reported in the previous studies. The two types of samples (specific and representative) used in this study showed similarity in the results for the all parameters examined (see section 5.1.1). This indicated that the sampling model used in this study is very credible and precise. The comparison between the results of six different plantations used as a samples source for this study, revealed significant variations between plantations located in western region and eastern region. However, the variations between the plantations within the same region are statistically not significant (see section 5.1.2).

The effect of different picks on the physiochemical properties of *Acacia* gum was examined using samples collected from different picks in both regions in Sudan. The effect on protein content, viscosity and molecular weight and molecular weight distribution was statistically significant (see section 5.2.1). In both regions, the first pick gave high values of % protein, viscosity, M_w and % AGP and then significantly decreased thereafter to the fourth pick.

The effect of tree age on *Acacia* gum characterisations was also examined in this study using samples collected from trees of varying age (5-20 years) from both regions. There is a significant variation in some physiochemical properties in correlation with the different

ages of tree in the Western region, where, samples collected from 15 years old trees gave considerably high viscosity and M_w gum compared with the young trees 5 years old and old trees 20 years (see section 5.2.2). This conclusion is in ~~a~~ good agreement with the conclusion of previous study (Idris et al., 1998). In the eastern region, an obvious trend similar to the western region is observed. However, the effect of different ages of trees on *Acacia* gum properties is statistically not significant (See section 5.2.2).

Generally, gums collected from western region showed significantly higher protein content, viscosity, molecular weight and % AGP fraction compared to gum collected from eastern region (see section 5.3). The variations in the gum properties between the two regions can be attributed to the differences in the environmental factors such as soil type, rainfall, temperature and humidity. Using the statistical method (regression analysis) the influence ~~s~~ and correlation of each of those factors on *Acacia* gum properties were examined (see section 5.2.3). The results revealed that the soil type and the average rainfall are the most influential factors that cause significant variation in physiochemical properties between the two regions. Where the sand soil showed strong positive correlation with all examined properties. Contrariwise the average rainfall showed a negative correlation with all the examined properties. Average temperature has negative correlation with the % protein and positive correlation with viscosity, M_w and % AGP. Additionally, the average relative humidity has negative correlation with % protein, viscosity and slightly positive correlation with M_w and % AGP. The regression analysis results suggested different models of equations allowed to some limit of certainty prediction of physiochemical properties of *Acacia* gum depending on different environmental factors. For some physiochemical properties, the model is statistically significant.

Subsequently, the influence of the physiochemical properties on the emulsification performance was examined by applying the same statistical analysis. Both parameters used

in this analysis (i.e. VMD and % volume of droplet greater than 1micron) confirmed that % AGP and viscosity are the most influential factors on emulsification performance (see section 7.1).

Our studies were extended to examine the effect of storage conditions on gum properties, in particular the molecular weight and molecular weight distribution. This was done by storing the samples in three environmentally different locations for a period of 5 years. Generally, the results showed that all stored samples demonstrated an increase in the M_w and in the % AGP consequently, showed an increase in emulsification performance. However, the statistically significant increase observed only in sample stored in locations with high temperature or/ and high humidity.

8.2 Suggested future work

Variation of the physiochemical properties and functional properties as function of different locations was observed. In particularly the influence of the soil type (sand and clay) as reported in section 7.1. A possible further study in soil minerals analysis to establish whether the minerals content in the different type of soil influence the physiochemical and functional properties of *A. senegal* gum or not.

Gum collected from different picks during the same season and from the same location also showed significant difference in the physiochemical properties of *Acacia* gum as reported in (5.2.1). Therefore, require more study consider with sugar content and amino acid profile for each separate pick to identify the influential factors. Additionally, more studies in gummosis and *Acacia* trees morphology are required to establish the factors that influences the gum exudation from pick to another.

We found in chapter 6 that the storage of the gum in specific locations for a period of 5 years led to a significant increase in both molecular weight and the mass of the high

molecular weight fraction in *A.senegal* gum (% AGP). However, further study required in the storage impact on *A.senegal* gum to establish precisely when the significant change in M_w value and % AGP start and for how long the changes is last.

Having reported the variation in the emulsification performance of acacia gum (chapter 7) due to the effect of different factors using acceleration test with VMD and Volume % droplets < 1 μm as parameters. Further study required in *Acacia* gum emulsification performance using long term stability test.

9. References

Abdel Rahman, H. M. (2001). *Effect of some growth parameters and age on gum and seed yield of Acacia senegal plantation and natural stand in north Kordofan*. Khartoum-Sudan.

Adiotomre, J., Eastwood, M. A., Edwards, C. A., & Gordon Brydon, W. (1990). Dietary fiber: In vitro methods that anticipate nutrition and metabolic activity in humans. *American Journal of Clinical Nutrition,* **52**, 128–134.

Akiyama, Y., Eda, S., & Kato, K. (1984). Gum arabic is a kind of arabino galactan protein. *Agric. Biol. Chem.,* **48**, 235 - 237.

Al-assaf, S. (2016). ANALYTICAL TECHNIQUES FOR THE CHARACTERIZATION OF POLYSACCHARIDES In S. Al-assaf (Ed.), *The radiation chemistry of polysaccharides*. Vienna: International Atomic Energy Agency.

Al-assaf, S., Andres-Brul, M., Cirre, J., & Phillips, G. O. (2012). Structural changes following industrial processing of *Acacia* gums. In F. John, Kennedy,, O. P. Glyn, & P. A. Williams (Eds.), *Gum Arabic* (pp. 153-168). UK: Royal Society of Chemistry.

Al-assaf, S., Katayama, T., Phillips, G. O., Sasaki, Y., & Williams, P. A. (2003). Quality control of gum arabic. *food hydrocolloids Jpn,* **2008**, 771-780.

Al-assaf, S., & Phillip, G. O. (2006). Characterisation of AGPs in Acacia gums. *Food & Food Ingred. J. Jpn,* **211**, 189-197.

AL-Assaf, S., & Phillips, G. O. (2009). Hydrocolloids Structure - Function Relationship. *Food science and Technology,* **23**, 17 - 20.

Al-Assaf, S., Phillips, G. O., Aoki, H., & Sasaki, Y. (2007). Characterization and properties of Acacia senegal (L.) Willd. var. senegal with enhanced properties (Acacia (sen) SUPER GUM™): Part 1—Controlled maturation of Acacia senegal var. senegal to increase viscoelasticity, produce a hydrogel form and convert a poor into a good emulsifier. *Food Hydrocolloids,* **21**(3), 319-328.

Al-Assaf, S., Phillips, G. O., & Williams, P. A. (2005). Studies on Acacia exudate gums: part II. Molecular weight comparison of the Vulgares and Gummiferae series of Acacia gums. *Food Hydrocolloids,* **19**, 661-667.

Amalvy, J. I. (1997). Dilute solution viscosimetry of carboxylated acrylic lactices *Pigments and Resin Technology* (**Vol. 26**, pp. 363-369). Argentina: MCB University Press.

Anderon, D. M. W., & Rahman, S. (1967). The viscosity-molecular weight relationship for Acacia gums. *Carbohydrate Polymers,* **4**, 298-304.

Anderson, D. M. W., Ashby, P., Busuttil, A., Eastwood, M. A., Hobson, B. M., Ross, A. H., & Street, C. A. (1982). *Subchronic effects of gum arabic (Acacia) in the rat*.

Anderson, D. M. W., & Dea, I. C. (1969). Light-scattering studies of Acacia gum exudates. *Carbohydrate research,* **10**, 161-164.

Anderson, D. M. W., Dea, I. C. M., Karamalla, K. A., & Smith, J. F. (1968). Studies on uronic acid materials : Part XXIV. An analytical study of different forms of the gum from Acacia senegal willd. *Carbohydrate research,* **6**, 97-103.

Anderson, D. M. W., Hirst, S. E., & Rahman, S. (1967). Studies on Uronic acid materials. *Carbohydrate research,* **3**, 308-317.

Anderson, D. M. W., & Smith, R. (1967). Studies on uronic acid materials : Part XIX. The composition of the gum from acacia laeta var. hashab. *Carbohydrate research,* **4**, 55-62.

Anderson, D. M. W., Stoddart, J. F., & Hirst, S. E. (1966). Some structural features of Acacia senegal gum (gum arabic). *Journal of the chemical society,* **C**, 1959 - 1966.

Aoki, H., Al-Assaf, S., Katayama, T., & Phillips, G. O. (2007). Characterization and properties of Acacia senegal (L.) Willd. var. senegal with enhanced properties (Acacia (sen) SUPER GUM™): Part 2—Mechanism of the maturation process. *Food Hydrocolloids,* **21**(3), 329-337.

Arman, M., & Qader, S. A. U. (2012). Structural analysis of kappa-carrageenan isolated from Hypnea musciformis (red algae) and evaluation as an elicitor of plant defense mechanism. *Carbohydrate Polymers,* **88**(4), 1264-1271.

Aspinall, G. O. (1970). *Polysaccharides*. New York: Pergamon.

B. Chikamai., M. Muga, & Marangu., M. (2012). Development of the gum Arabic sub-sector in Kenya. In F. John, Kennedy,, Glyn O. Phillips, & P. A. Williama. (Eds.), *Gum Arabic*. Cambridge, UK: The Royal Society of Chemistry.

Ballal, M. E., El Siddig, E. A., Elfadl, M. A., & Luukkanen, O. (2005). Gum arabic yield in differently managed Acacia senegal stands in western. *Agroforestry Forum*, **63**, 237–245.

Ballal, M. E., El Siddig, E. A., Elfadl, M. A., & Luukkanen, O. (2005). Relationship between environmental factors, tapping dates, tapping intensity and gum arabic yield of an Acacia senegal plantation in western Sudan. *Journal of Arid Environments,* **63**, 379-389.

Benichou, A., Aserin, A., & Garti, N. (2004). Double emulsions stabilized with hybrids of natural polymers for entrapment and slow release of active matters. *Advances in Colloid and Interface Science,* **108-109**, 29-41.

Bentham, G. (1842). Notes on Mimoseae, with a synopsis of species. *London Journal of Botany*, **1**, 318–528.

Bentham, G. (1864). *Acacia*. *In , Flora Australiensis* (**Vol. 2**). London: Lovell Reeve.

Bentham, G. (1875). Revision of the suborder Mimoseae. *Transactions of the Linnean Society of London,* **30**, 335–664.

Beyer, M., Reichert, J., Heurich, E., Jandt, K. D., & Sigusch, B. W. (2010). Pectin, alginate, and gum arabic polymers reduce citric acid erosion effects on human enamel. *Dental Materials,* **26**, 831-839.

Bliss, D. Z., Stein, T. P., Schleifer, C. R., & Settle, R. G. (1996). Supplementation with gum arabic fiber increases fecal nitrogen excretion and lowers serum urea nitrogen in chronic Walter consuming a low-protein diet. *American Journal of Clinical Nutrition,* **63**, 392-398.

Braun, D., H. Cherdron, M. Rehahn, H. Ritter, & Voit., B. (2004). *Polymer Synthesis: Theory and Practice* (4 ed.). New York: Springer Berlin Heidelberg.

Breuer, M. M. (1985). Cosmetic Emulsions. *Encyclopedia of Emulsion Technology Becher, P. (Ed.), Marcel Dekker: New York,* **3**, 386.

Brown, I., A. (2011). The physiological roles of dietary fibre. *Food Hydrocolloids,* **25**, 238-250.

Brucker, C., Uhlarik, K. S., Lampe, J. W., & Bush, J. W. (1974). US Patent No. 3,800,045.

Buffo, R. A., Reineccius, G. A., & Oehlert, G. W. (2001). Factors affecting the emulsifying and rheological properties of gum acacia in beverage emulsions. *Food Hydrocolloids*, **15**, 53-66.

Burchard, W. (1994). (S.B. Ross-Murphy, Ed) Glasgow.

Caius, J. F., Radha, K., & Bombay, J. (1939). *Nat*. *Hist*. *Soc,* **41**, 261.

Castellani, O., Gaillard, C., Vié, V., Al-Assaf, S., Axelos, M., Phillips, G. O., & Anton, M. (2010). Hydrocolloids with emulsifying capacity. Part 3 – Adsorption and structural properties at the air–water surface. *Food Hydrocolloids,* **24**(2-3), 131-141.

Castellani, O., Guibert, D., Al-Assaf, S., Axelos , M., Phillips, G. O., & Anton, M. (2010). *Food Hydrocolloids*, **24**, 193-199.

Chawla, R., & Patil. (2010). Soluble dietary fibre. *Comperhensive Reviews in Food Science and Food Safety*, **9**, 178-196.

Cherbut, C., Michel, C., Raison, V., Kravtchenko, T., & Meance, S. (2003). Acacia gum is a bifidogenic dietary fibre with high digestive tolerance in healthy humans. *Microbial Ecology in Health and Disease,* **15**, 43-50.

Cherukuri, S., Friello, D. R., Parker, E., Hopkins, W., & Mackay, D. (1983). US Patent No. 4,371,549.

Chikamai, B. N., Banks, W. B., & Anderon, D. M. (1996). Processing of gum arabic and some new opportunities. *Food Hydrocolloids*, **10**, 309-316.

Chikami, B. N. (2001). *Food ingredient for the future*. Tunisia.

Churms, S. C., Merrifield, E. H., & Stephen, A. M. (1983). Some new aspects of the molecular structure of Acacia senegal gum (gum arabic). *Carbohydrate research*, **123**, 267-279.

Connolly, S., Fenyo, J., & Vandevelde, M. (1987). Heterogeneity and homogeneity of an arabinogalactan-protein: Acacia senegal gum. *Food Hydrocolloids*, **1**, 477-480.

Cui, S. W. (2005). *Food Carbohydrates Chemistry, Physical properties and applications*. New York: Taylor & Francis Group.

Daniel, T., Piorkowski, Julian, D., & McClements. (2014). Beverage emulsions: Recent developments in formulation, production, and applications. *Food Hydrocolloids*, **42**, 5-41.

David, G. A., Kazunari, U., & Aled, P. (2009). Acacia(sen) SUPERGUM (Gum arabic): An evaluation of potential health benefits in human subjects. *Food Hydrocolloids*, **23**, 2410–2415.

Dickinson, Eric. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, **17**, 25-39.

Dickinson, E. (2010). Double Emulsions Stabilized by Food Biopolymers. *Food Biophysics*, **6**(1), 1-11.

Dickinson, E., & Galazka, V. B. (1992). Emulsion stabilization by protein/ polysaccharides complex. *Gum and stabilisers for the food industry*, **6**, 351-362.

Dickinson, E., Galazka, V. B., & Anderson, D. M. W. (1991). Emulsifying behaviour of gum arabic. Part 1: Effect of the nature of the oil phase on the emulsion droplet-size distribution. *Carbohydrate Polymers*, **14**, 373-383.

Dickinson, E., Murray , B., Stainsby, G., & Anderson, D. M. W. (1988). Surface activity and emulsifying behaviour of some Acacia gums. *Food Hydrocolloids*, **6**, 477-490.

Doi, Y., Ichihara, T., Hagiwara, A., Imai, N., Tamano, S., Orikoshi, H., . . . Shirai, T. (2006). A ninety-day oral toxicity study of a new type of processed gum arabic, from Acacia tree (Acacia senegal) exudates, in F344 rats. *Food and Chemical Toxicology*, **44**, 560-566.

Duvallet, S., Fenyo, J., & Vandevelde, M. (1989). Meaning of molecular-weight-measurments of gum arabic. *Polymer Bulletin*, **21**, 517 - 521.

Duvallet, S., Fenyo, J. C., & Vandevelde, M. C. (1993). The characterization of gum arabic from an experimental field of Ferlo (North Senegal). *Food Hydrocolloids*, **7**, 319-326.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing characterisation, technological, functionality and commercial applications: A review. *Food Chemistry,* **124**, 411-421.

Elmanan, M., Al-Assaf, S., Phillips, G. O., & Williams, P. A. (2008). Studies on Acacia exudate gums: Part VI. Interfacial rheology of Acacia senegal and Acacia seyal. *Food Hydrocolloids,* **22**, 682-689.

Etacheri, V., Marom, R., ELazari, R., Salitra, G., & Aurbach, D. (2011). Challenges in the development of advanced Li-ion batteries: a review *Energy Environ. sci,* **4**, 3243.

Evans, M., Ratcliffe, I., & Williams, P. A. (2013). Emulsion stabilisation using polysaccharide–protein complexes. *Current Opinion in Colloid & Interface Science,* **18**(4), 272-282.

FAO/WHO. (1982). *TOXICOLOGICAL EVALUATION OF CERTAIN*. Rome.

FAO/WHO. (1990). *Toxicological evaluation of certain food additives and contaminants*. Geneva.

FAO/WHO. (1995). *Gums, resins and latexes of plant origin*. Rome.

FAO/WHO. (2001). *Food standards programme*. Geneva 24.

FAO/WHO. (2003). *Food standards programme*. Rome 25.

FAO/WHO. (2004). *Food standards Programme*. Geneva 27.

FAO/WHO. (2005). *Food standard programe*. Rome 28.

FAO/WHO. (2009). *Food standards programme*. Rome 32.

FDA. (1974). *Proposed affirmation of GRAS status for gum arabic. Federal Register* (39).
Ferdinand, G., & Kruger, W. (1986). Germany Patent No. 3, 517, 916.

Fincher, Stone, & AE, C. (1983). Arabinogalactan-proteins: structure, biosynthesis, and function. *Annu Rev Plant Physiol*(34), 47–70.

Fuyama, H., & Tsuji, K. (1981). U.S Patent No. 4,283,415.

GAB. (2014). *The annual exported quantities of gum Arabic* Sudan.

GAC. (2009). *Annual reports of Gum Arabic producation and quality in Sudan* Sudan.

Gamble, D. L., & Grady, L. D. (1938). U.S Patent No. 2,135,936.

Gibson, G. R., & Roberfroid, M. B. (2013). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Nutrition,* **125**, 1401-1412.

Gidley, M. J. (2013). Hydrocolloids in the digestive tract and related heaith implications. *Current Opinion in Colloid & Interface Science*, **18**, 371-378.

Glicksman, M. (1983). *Food Hydrocolloids*. Boca Raton, USA: CRC Press, .

Glicksman, M., Sand, R. E., & Whistler, R. L. (1973). Gum Arabic *Industrial Gums* (pp. 197-263): Academic Press.

Godlewski, M., & Adamczyk, B. (2007). The ability of plants to secrete proteases by roots. *Plant Physiol Biochem*, **45**, 657-664.

Goodrum, L. J., Leykam, J. F., & Kieliszewski, M. J. (2000). Gum arabic glycoprotein contains glycomodules of both extensin and arabinogalactan-glycoproteins *phytochemistry*, **54**, 99-106.

Gray, J. (2006). *Dietary fibre. Definition, analysis, physiology and health*.

Griffin, W. C. (1949). Classification of surtace-Active Agent by HLB. *Cosmetic chem*, **1**, 311-326.

Hassan, R. A. (1999). Investigation of Stem Cutting Propagation and Factors Affecting Gum Production of Acacia senegal (L) WILD. *Department of Siliviculure, Faculty of Forestry, University of Khartoum*.

Hayashi, H. (2002). Japan Patent No. 1302212; PCT-JP02/08144; WO03/093324 A1

Hayward, B. (2004). *Acacia tree; a sustainable resource for Africa*. UK.

Henson, W. A., & Westveer, W. M. (1956). U.S Patent No. 2,758,103.

Hipsley, E. H. (1953). Dietary "fibre" and pregnancy toxaemia. *British medicalJournal*, **2**, 420-422.

Huggins, M. L. (1942). The viscosity of dilute solution of long chain mo;ecules. IV Dependence on the concentration *Journal of the American Chemical Society*, **64**, 2716-2718.

Idris, O., Williams, P. A., & Phillips, G. O. (1998). Characterisation of gum from Acacia senegal trees of different age and location using multidetection gel permeation chromatography. *Food Hydrocolloids*, **12**, 379-388.

Idris, Y. M. A., Ibrahim, Y. A., & Mariod, A. A. (2013). Colour dehydrated tomato: Effect of gum arabic. *International Journal of Food Properties*, **16**.

IIED, & IES. (1990). *Gum Arabic rehabilitation in the republic of the Sudan* Khartoum.

Imeson, A. (1992). *Exudate gums. In thicking and gelling agents for Food*. London.

Iserbyt, P., Schouppe, G., & Charlier, N. (2015). A multiple linear regression analysis of factors affecting the simulated Basic Life Support (BLS) performance with Automated External Defibrillator (AED) in Flemish lifeguards. *Resuscitation*, **89**(0), 70-74.

Islam, Phillip, G. O., Sljivo, M., & Williams, P. (1997). A review of recent developments on the regulatory, structural and functional aspect of gum arabic. *Food Hydrocolloids*, **11**, 493-505.

Itthivadhanapong, P., Jantathai, S., & Schleining, G. (2016). Improvement of physical properties of gluten-free steamed cake based on black waxy rice flour using different hydrocolloids. *J food Sci Technol*, **53**(6), 2733-2741.

JACKSON, C., & BARTH, H. G. (2004). *"Molecular weight sensitive detectors for size exclusion chromatography", Handbook of Size Exclusion Chromatography and Related Techniques* (C.-S. WU Ed. 2nd ed ed.). New York, NY Marcel Decker.

JCEFA-FAO. (1999). FOOD AND NUTRATION PAPER. *FNP52*, **7**.

JECFA-FAO. (1997). *Limit test for heavy metals in food additive specifications*. Rome.

JECFA-FAO. (1999). *Food and Nutrition paper FNP*. Roma.

John, F., Kennedy,, Phillips, G. O., & Williams, P. A. (2012). *Gum Arabic*. Cambridge UK: The Royal Society of Chemistry.

Joseleau, J. P., & Ullmann, G. (1990). Biochemical evidence for the site of formation of gum Arabic in acacia senegal. *phytochemistry*, **29**, 3401-3405.

Jurasek, P., Kosik, M., & Phillips, G. O. (1993). A chemometric study of the Acacia (gum arabic) and related natural gums. *Food Hydrocolloids*, **7**, 73-85.

Kandethody M. Ramachandran., & Tsokos., C. P. (2009). *Mathematical statistics with Applications*. USA: ELsevier Acadmemic Press.

Karamalla, K. A., Siddig, N. E., & Osman, M. E. (1998). Analytical data for Acacia senegal var. senegal gum samples collected between 1993 and 1995 from Sudan. *Food Hydrocolloids*, **12**, 373-378.

Kaur, A., Shevkani, K., Singh, N., Sharma, P., & Kaur, S. (2015). Effect of guar gum and xanthan gum on pasting and noodle-making properties of potato, corn and mung bean starches. *J food Sci Technol*, **52**(12), 8113-8121.

Kendall, C. W. C., Esfahani, A., & Jenkins, D. J. (2010). The link between dietary fibre and human health. *Food Hydrocolloids*, **24**, 42-48.

Kishimoto, A., Ushida, K., Phillips, G. O., Takashi Ogasawara, T., & Sasaki, Y. (2006). Identification of intestinal bacteria responsible for fermentation of gum Arabic. *Current Microbiology*, **53**, 173–177.

Kralova, I., & Sjöblom, J. (2009). Surfactants Used in Food Industry: A Review. *Journal of Dispersion Science and Technology,* **30**(9), 1363-1383.

Lam, L., & Teng, J. (2002). Strength Models for fiber-reinforced plastic-confined concrete. *J. Struct. Eng,* **128:5**, 612-623.

Lane, E. (1957). Spectrophotometric and turbidimetric methods for measuring proteins. *Methods Enzymol,* **13**, 447-453.

Lapasin, R., & Pricl, S. (1995). The polysaccharides: sources and structures *Rheology of Industrial Polysaccharides: Theory and Applications* (pp. 1-133). Boston, MA: Springer US.

Laurier, L. S. (2005). *Emulsions, Foams, and Suspensions: Fundamentals and Applications*. Weinheim- Germany: WILEY-VCH Verlag GmbH & Co. KGaA.

Lipson, D., & Nasholm, T. (2001). The unexpected versatility of plants: organic nitrogen ude and availability in terrestrial ecosystem. *Oecologia,* **128**, 305-316.

Liu, R. X., Kuang, J., Gong, Q., & Hou, X. L. (2003). Principal component regression analysis with spss. *Computer Methods and Programs in Biomedicine,* **71**(2), 141-147.

Lowry, O., Rosbrough, N., Farr, A., & Randall, R. (1951). The Lowry assay -general use protein assay. *J. Biol. Chem193,* **193**.

Mahendran, T., Williams, P., Phillips, G. O., Al-assaf , S., & Baldwin, T. C. (2008). New insights into the structural characteristics of the Arabinogalactan protein (AGP) fraction of gum Arabic. *Agric. Food Chem,* **56**, 9269-9276.

Maqbool, M., Ali, A., Alderson, P. G., Zahid, N., & Siddiqui, Y. (2011). Effect of a novel edible composite coating based on gum arabic and chitosan on biochemical and physiological responses of banana fruits during cold storage. *Journal of agricultural food chemistry,* **59**, 5474-5482.

Maslin, B. R., Miller, J. T., & Seigler, D. S. (2003). Overview of the generic status of Acacia (Leguminosae: Mimosoideae). *Australian Systematic Botany,* **16(1)**, 1-18.

Matthias, S. (2010). *Gum and stabilisers for the food industry*. Cambridge.

McCleary, B., V. (2003). Dietary fibre analysis. *Proceedings of the Nutririon Society,* **62**, 3-9.

McCleary, B., V, DeVries, J., W, Rader, J. I., Gohen, G., Prosky, L., & Mungford, D., C. (2012). Determination of insoluble, soluble, and total dietary fibre (CODEX definition) by enzymatic-gravimetric methods and liquid chromatography: Collaborative study. *AOAC International,* **95**, 824-844.

McClements, D. (1999). *Food emulsions*: Boca Raton: CRC Press.

McClements, D. J. (2002). Theoretical prediction of emulsion color. *Advances in Colloid and Interface Science*, **97**, 63-89.

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science*, **9**(5), 305-313.

McClements, D. J., & Li, Y. (2007). Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. *Advances in Colloid and Interface Science*, **159**(2), 213-228.

McLean Ross, A. H., Eastwood, M. A., Brydon, W. G., Anderson, J. R., & Anderson, D. M. (1983). A study of the effects of dietary fibre gum arabic in humans. *American Journal of Clinical Nutrition*, **37**, 368-375.

McLean Ross, A. H., Eastwood, M. A., Brydon, W. G., Busuttil, A., & McKay, L. F. (1984). A study of the effects of dietary gum arabic in the rat. *British Journal of Nutrition*, **51**, 47–56.

McNamee, Riordan, O., & Sullivan, M. (1998). Emulsification and microencapsulation properties of gum arabic. *Agric Food Chem*, **46**, 4551-4555.

Meng, G., Xing-cheng, X., Gao, L., Suntharampillai, T., Donald, R. B., Ji-Guang, Z., . . . Chong-Min, W. (2014). Mesoscale Origin of the Enhanced Cycling-Stability of the Si-Conductive Polymer Anode for Li-ion Batterie. *Scientific Reports*, **4**, 3684.

Metha, R. (2009). Dietary fibre. *AIB International Technical Bulletin*, **XXXI**, 1-7.

Metz, H. C. (1991). *Sudan*. Washington.

Milani, J., & Maleki, G. (2012). *Food Idustrial Processes-Methods and Equipment*. China: In Tech.

Millard, R., & Balmert, C. A. (1961). U.S Patent No. 2, 985, 562.

Min, L., Yana, X., Hui, Z., Xingxing, G. U., Jingxia, Q., Sheng, L., . . . Shanqing, Z. (2015). Dual-functional gum Arabic binder for silicon anodes in lithium ion batteries. *Nano Energy*, **12**, 178-185.

Miyamoto, Kotake, T., Boncela, A. J., Saniewski, M., & Ueda, J. (2015). Hormonal regulation of gummosis and composition of gums from bulbs of hyacinth (Hyacinthus orientalis). *J Plant Physiol*, **174**, 1-4.

MOED.,(Ministry of Environment development- Sudan) (2003). *Convention on Climate Change*. Sudan.

Mora, Y. N., Contreras, J. C., Aguilar, C. N., Meléndez, P., Ileana De la Garza, & Rodríguez, R. (2013). Chemical Composition and Functional Properties from Different Sources of Dietary Fiber. *American Journal of Food and Nutrition*, **1**(3), 27-33.

Moreira, M. R., Cassani, L., Martin-Belloso, O., & Soliva-Fortuny, R. (2015). Effects of polysaccharide-based edible coatings enriched with dietary fiber on quality attributes of fresh-cut apples. *J food Sci Technol,* **52**(12), 7795-7805.

National research council, A. (2005). *Dietary reference intakes for energy, carbohydrate, fibre, fat, fatty acid, cholesterol, protein, and amino acid (macronutrients)*. Washington, DC.

Navon-Venezia, Zosim, S., Gottlieb, A., Legmann, R., Carmeli, S., Ron, E., & Rosenberg, E. (1995). A new bioemulsifier. *Applied and Environment Microbiology,* **61**, 3240-3244.

Nie, S.-P., Cui, S. W., Wang, Q., Xie, M.-Y., & Phillips, G. O. (2012). A further amendment to the classical core structure of gum arabic (Acacia senegal). *Food Hydrocolloids,* **31**, 42-48.

Onishi, T., Umemura, S., Yanagawa, M., Matsumura, M., Sasaki, Y., Ogasawara, T., & Ooshima, T. (2008). Remineralization effects of gum arabic on caries-like enamel lesions. *Archives of Oral Biology,* **53**, 257-260.

Osman, A. A., Osman, M. E., Hassan, E. A., Al-assaf, S., Andees-Brul, M., & Phillip, G. O. (2012). Characterisation, fractionation and emulsification properties of Acacia Polyacantha Gum. In F. john, Kennedy,, Glyn O. Phillips, & P. A. Willams. (Eds.), *Gum Arabic* (pp. 209-217). Camberidge The Royal Socity of Chemistry.

Osman, M. E., Menzies, A., Williams, P., & Phillip, G. O. (1994). Fractionation and characterization of gum arabic samples from various African countries. Food. *Food Hydrocolloids,* **8**, 233-242.

Osman, M. E., Menzies, A. R., Williams, P. A., Phillips, G. O., & Baldwin, T. C. (1993). The molecular characterisation of the polysaccharide gum from Acacia senegal. *Carbohydrate research,* **246**, 303-318.

Pedley, L. (1978). *A revision of Acacia Mill*. in Queensland.

Pedley, L. (1986). Derivation and dispersal of Acacia (Leguminosae), with particular reference to Australia, and the recognition of Senegalia and Racosperma. *Botanical Journal of the Linnean Society,* **92**, 219–254.

Pedley, L. (1987a). In defence of Racosperma. *Bulletin of the International Group for the Study of Mimosoideae,* **15**, 123–129.

Pedley, L. (1987b). Racosperma deltoideum (Cunn. ex G. Don) Pedley (Leguminosae: Mimosoideae) and related species on northern Australia. *Austrobaileya,* **2(4)**, 314–320.

Pedley, L. (1989). Racosperma again. *Australian Systematic Botany Newsletter*, **59**, 1-2.

Peterson, G. (1979). Review of Folin phenol method of Lowry. *Anal Biochem,* **100**, 201-220.

Peterson, G. (1983). Determination of total protein. *Methods Enzymol,* **91**, 95-119.

Phillips, & Williams, P. A. (2009). *Handbook of Hydrocolloids* (2 ed.): Woodhead Publishing.

Phillips, G. O., Ogasawara, T., & Ushida, K. (2008). The regulatory and scientific approach to defining gum arabic (Acacia senegal and Acacia seyal) as a dietary fibre. *Food Hydrocolloids,* **22**, 24-35.

Qi, W., Fung, C., & Lamport, D. T. (1991). Gum arabic glycoprotein is a twisted hairy rope. A new model based on O-galactosylhydroxyproline as the polysacchairde attachment site. *Plant Physiol,* **96**, 848 - 855.

Ramírez, J. A., Uresti, R. M., Velazquez, G., & Vázquez, M. (2011). Food hydrocolloids as additives to improve the mechanical and functional properties of fish products: A review. *Food Hydrocolloids,* **25**(8), 1842-1852.

Randall, R., Phillips, G. O., & Williams, P. A. (1988). The role of the proteinaceous component on the emulsifying properties of gum arabic. *Food Hydrocolloids,* **2**, 131-140.

Randall, R. C., Phillips, G. O., & Williams, P. A. (1989). Fractionation and characterization of gum from Acacia senegal. *Food Hydrocolloids,* **3**, 65-75.

Redgwell, R. J., & Fischer, M. (2005). Dietary fibre as versatile food component: An industrial perspective. *Molecular Nutrition and Food research,* **49**, 521-535.

Rinaudo, M., Auzely, R., & Mazeau, K. (2004). *Encyclopedia of Polymer Science and Technology*. France: Joeseph Fourier University.

Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: a critical review. *J food Sci Technol,* **47**(6), 587-597.

Samaan, M., Mirmiran, A., & Shahawy, M. (1998). Model of Concrete Confined by Fiber Composites. *J. Struct. Eng,* **124:9**, 1025-1031.

Sanchez, C., Schmitt, C., Kolodziejczyk, E., Lapp, A., Gaillard, C., & Renard, D. (2008). The Acacia Gum Arabinogalactan Fraction Is a Thin Oblate Ellipsoid: A New Model Based on Small-Angle Neutron Scattering and Ab Initio Calculation. *Biophysical* **94**, 629-639.

Schultz, C. J., Johnson, K. L., Currie, G., & Basic, A. (2000). The classical arabinogalactan protein gene family of arabidopsis. *Plant Cell,* **12**, 1751-1767.

Seema, P., & Arun, G. (2015). Application of natural polymer Gum Arabic. *International Journal of Food Properties,* **18:5**, 986-998.

Shama, R. D. (1985). Hypoglycemic effect of gum acacia in healthy human subjects. *Nutrition Research,* **5**, 1437–1441.

Sherrier, D. J., Prime, T. A., & Dupree, P. (1999). Glycosylphosphatidylinositol-anchored cell-surface protein from Arabidopsis. *Electrophoresis*, **20**, 2027-2035.

Shidi, X., Bin, X., Andrew, M., Vince, B., & Gao, L. (2013). Conductive Polymer and Silicon Composite Secondary Particles for a High Area-Loading Negative Electrode. *Electrochem. Soci*, **160**, 1380-1383.

Shigeo, O., Hiroya, Y., Sumio, T., & Keiji, M. (1989). European Patent No. 10,334,466.

Skrzypek, Miyamoto, K., Saniewski, M., & Ueda, J. (2005). Jasmonates are essential factors inducing gummosis in tulips: mode of action of jasmonates focusing on sugar metabolism. *J Plant Physiol*, **162**(5), 495-505.

Slavin, J. (2003). Why whole grains are protective: Biological mechanisms. *Proceedings of the Nutririon Society*, **62**, 129-134.

SMA., (Sudan Merology Authority) (2013). *Crop Insurance*. Sudan.

Smith, F., & Montgomery, R. (1959). *The chemistry of plant gums and Mucilage's* New York: Rein hold publishing corporation.

Sperling, L. H. (2006). *Introduction to Physical Polymer Science* (4th edn ed.). New York: John Wiley & Sons.

Stamler, J., Stamler, R., & Neaton, J. D. (1993). Blood pressure, systolic and diastolic, and cardiovascluar risk. *U.S. population data. Archives of InternalMedicine*, **153**, 598-615.

Stephen, M., A., Phillips, G. O., & Williams, P. A. (2006). *Food Polysaccharides and their application*. New York: Taylor & Francis Group.

Stephen, A. M., & Churms, S. C. (1995). *Food polysaccharides and their application*. New York: Marcel Dekker, Inc.

Street, C. A., & Anderson, D. M. W. (1983). Refinement of structures previously proposed for gum arabic and other acacia gum exudates. *Talanta*, **30**, 887-893.

Svetec, J., Yadav, M. P., & Nothnagel, E. A. (1999). Presence of aglycosylphosphatidylinositol lipid anchor on rose arabinogalactan proteins. *Biol Chem*, **274**, 14724-14733.

Tame-Said, J. I. (1997). World Patent Patent No. 9,719,668,1997.

Thevenet, F. (1988). *Acacia gums-stabilizers for flavor encapsulation*. Washington

Tiss, A., Carriere, F., & Verger, R. (2001). Effects of gum arabic on lipase interfacial binding and activity. *Anal Biochem*, **294**, 36-43.

Trowell, H. C., Southgate, D., J, A, Wolever, T., M, S, Leeds, A., R, Gassull, M., A, & Jenkins, D., J, A. (1976). Dietary fibre redefined. *Lancet*, **2**, 1408-1412.

UNCTAD. (2013). Infocomm- commodity profile Gum Arabic http://www.unctad.info.

Vandevelde, M.-C., & Fenyo. (1985). Macromolecular distribution of Acacia senegal gum (gum arabic) by size-exclusion chromatography. *Carbohydrate Polymers*, **5**, 251-273.

Vassal, J. (1972). Apport des Recherches ontoge´nigues et seminologiques a l'e´tude morphologique taxanomique et phyloge´nique de genre Acacia. *Bulletin de la Societe d'Histoire Naturelle de Toulouse,* **108**, 105–247.

Verbeken, D., Dierckx, S., & Dewettinck. (2003). Exudate gums: Occurrence, production and application. *Applied Microbiology and Biotechnology*, **63**, 10-21.

Viebke, C., Al-Assaf, S., & Phillips, G. O. (2014). Food hydrocolloids and health claims. *Bioactive Carbohydrates and Dietary Fibre*, **4**(2), 101-114.

Walstra, P., & Smulders, P. A. (1998). *Modern aspects of emulsion sceince*.

Weigelt, A., King, R., Bol, R., & Bardgett, R. (2003). Inter-specific variabilityin organic nitrogen uptake. *J Plant Nutr Soil Sci*, **166**, 606-611.

Welsh, T. F., Black, J. J., Graham, T. N., & Brown, L. H. (1987). Study of the teratogenic potential of gum arabic. *Food and Chemical Toxicology*, **25**, 815-821.

Whistler, R. (1993). *Polysaccharides and their derivatives*. San Diego, USA: Acadwmic Press, San Diego.

Williams, P. A., & Langdon, M. J. (1995). *Chromatography and Analysis*.

Williams, P. A., & Phillip, G. O. (2000). *Handbook of hydrocolloids* (P. A. Williams Ed.). Cambridge, UK: CRC Press.

Yadav, M. P., Johnston, D. B., Hicks, K. B., & Nothnagel, E. A. (2006). The role of lipid and protein components in the emulsification properties of gum arabic and corn fibre gum. *Food and Food Ingredients Journal of Japan*, **211**, 243-254.

Youl, J. J., Basic, A., & Oxley, D. (1998). Arabinogalactan-proteins from Nicotiana alata and Pyrus communis contain glycosylphosphatidylinositol membrane anchors. *Proc. Natl. Acad. Sci.USA*, 7921-7926.

Young, R. J., & Lovell, P. A. (1991). *Introduction to Polymers, Second Edition*: Taylor & Francis.